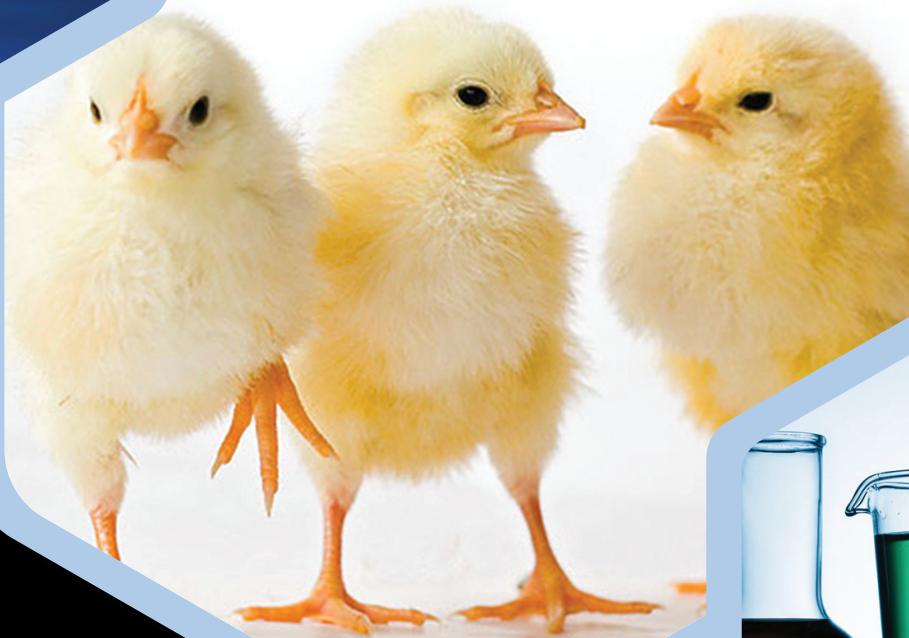


2022

INTERNATIONAL POULTRY SCIENTIFIC FORUM



JAN. 24 - 25

ABSTRACTS
2022 International Poultry Scientific Forum
Georgia World Congress Center, Atlanta, Georgia
January 24-25, 2022

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ABSTRACTS

2022 International Poultry Scientific Forum

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Dendy Keynote Lecture

KEYNOTE, COVID pandemic influences on broiler production Bruce Stewart-Brown* *Perdue Foods*

COVID has been devastating and fascinating. Many of us were basically trying to learn and react at the same time (maybe we still are in some ways). Chicken demand was changing, our relationship with the consumer was changing, the labor force was changing, and people were wondering if they needed to live in a different way in a different place.

COVID turned the poultry supply chain upside down. Eggs were coming, birds were growing, but plants were either struggling with labor or unpredictable demand. Some “once in a lifetime” type events happened on the live side during this period. Describing a few of these might help if I’m wrong about “once in a lifetime”.

Consumers and Customers want and demand a relationship with the companies providing them with food. COVID had a pretty dramatic effect on most company’s metrics associated with consumer inquiries. If a consumer wonders it, they can easily ask it. However, the consumer became a little less likely to complain.

COVID is pushing us to automation. One of the most labor-intensive areas of a harvest facility is the deboning department. If we are heading for more auto deboning, the process will demand a stronger focus on incoming flock weight and uniformity. The more uniform the bird presented to an auto-deboning machine, the better we can cleanly (and safely) remove the meat. I see no down side to understanding and measuring uniformity – no matter what the processing technology holds.

The COVID vaccines look to be some of the most fantastic vaccines ever invented. The development time was lightning fast (or seemed to be). The effectiveness is beyond almost anything we have ever seen. Our vaccines in poultry have been industry changing. But they are just OK. And.... they are generally very cheap. But...many of them cause mild disease. That was acceptable when birds were spread out. It was acceptable when placement to harvest was 50 days or more. We in the poultry industry need this jump in vaccine technology too.

COVID has made many people question where they live. Population density deserves more respect and analysis. We should (or will be asked to) look closely at appropriate animal density. When does an area have too many animals? As our poultry population density grew we have tried to enforce better biosecurity. Biosecurity (and common disease containment practices) are only so good. Unless they become very extreme (SPF). I don’t believe we can (or should) raise all our meat birds in an SPF housing type setup. When is a chicken house too crowded? When are there too many chicken houses in an area?

COVID variants are a hot topic for human medicine. We are “experts” at creating virus variants. We should work as hard at NOT creating variants as we do developing new vaccines for them.

Key Words: COVID

*Author presenting paper

GS Denotes Graduate Student Competition
UG Denotes Undergraduate Presentation

Physiology, Endocrinology and Reproduction: Layer or Broiler Breeders

M1 Cold storage after egg inoculation enhances *Campylobacter* recovery from embryos and egg contents thru day 5 of incubation Caitlin Harris^{*1,2GS}, Lydia Bartenfeld Josselson², Richard Buhr^{1,2} ¹University of Georgia, ²USDA-ARS, US National Poultry Research Center

Campylobacter spp. are important foodborne pathogens and increased knowledge on potential egg transmission would be beneficial for developing intervention strategies. Data is lacking for *Campylobacter* verifying vertical egg transmission compared to *Salmonellae*. The first objective of the experiment compared *Campylobacter* inoculation of albumen (1AL) or yolk (2YO) injected hatching eggs that were cold stored, then incubated. The second objective tested *Campylobacter* inoculation directly on the vitelline membrane (3VM) before incubating. 150 SPF White Leghorn eggs were collected and divided into 3 trt (n=40 1AL and 2YO; n=30 3VM). On d0 for 1AL and 2YO, 10³ CFU *C. coli* inoculum was injected into the albumen or yolk. For trt 1AL and 2YO, inoculated eggs were stored for 2d (6°C; 54%RH) and transferred to incubator for 5d (37.5°C; 55% RH). On d0 for 3VM, eggs were aseptically broken, albumen and yolk separated, and 10³ CFU *C. coli* placed onto vitelline membrane. After 5 min, albumen was added on top of the inoculated yolk and sample cups incubated for 5d (37.5°C; 55% RH). On each day of storage and incubation, 5 eggs/trt were sampled, 1:1 diluted using BPW, and direct and enriched plated in triplicate. On d5 of incubation, embryos were aseptically removed and sampled separately from egg contents. Results were recorded as positive or negative; Kruskal-Wallis test was used to determine significance (p≤0.05). On d0, all trt were 100%+ direct prior to cold storage or incubation. For both storage d, 1AL and 2YO samples were 100%+ enriched. For incubation d1 to 5 there was a significant difference between trt for *Campylobacter* recovery. 3VM samples were 0%+ enriched and 1AL were 0 to 20%+ enriched for d1 to 4. On d5, egg contents for 1AL and 2YO were 60%+ enriched and 3VM was statistically different with 0%+ enriched. On d5, *Campylobacter* recovery from embryos was 0%+ for 1AL and 3VM, which was significantly lower than 2YO recovery at 100%+ enriched. Overall, *Campylobacter* was recovered on all sample d for 2YO from egg contents and embryos, and recovery was lower for 1AL and 3VM. These results are the first to confirm that yolk inoculation, cold storage for 2d, and then incubation may be a possible method to study vertical transmission of *Campylobacter* during incubation.

Key Words: *Campylobacter*, egg inoculation, egg cold storage, egg incubation

M2 Effects of cumulative nutrient intake and growth parameters during rearing on the reproductive performance of broiler breeders under commercial conditions Maria Alfaro-Wisaquillo^{*1GS}, Gustavo Quintana-Ospina¹, Luis Bernal-Arango², Juan Ruiz-Ramirez², Gustavo Martínez-Bernal², Edgar Oviedo-Rondón¹ ¹North Carolina State University, ²Grupo BIOS Inc.

Cumulative nutrient intake (CNI), BW, and flock uniformity (FU) during rearing have been demonstrated to affect broiler breeders' reproductive results. However, the relationship between those factors and optimum CNI during specific weeks needs to be explored under commercial conditions. A study was conducted to detect the effects of the cumulative intake of energy (AMEn), crude protein (CP), digestible lysine (dLys), calcium (Ca), phosphorus (P), and fat, and their relationship with BW and FU from 1 to 24 wk of age in the reproductive performance of Ross 308 AP broiler

breeders at 55 wk under tropical commercial conditions. The evaluated database consisted of feed nutrient composition, growth, and reproductive performance records from 45 broiler breeder flocks raised between 2017 and 2020, representing 1,400,769 hens housed. Partition trees were used to classify breeder flocks as low (LP) or high (HP) performance according to the hen-housed egg production (HHEP) at 55 wk. Data were analyzed by a one-way ANOVA using the student's t-test for mean comparisons. Linear, quadratic, and multiple linear regressions (MLR) were fit to find relationships among BW, FU, and CNI from 1 to 24 wk with the HHEP at 55 wk of age. The HP group had higher ($P < 0.05$) CNI of all nutrients, except for Ca, than the LP group up to 24 wk. Cumulative intake of AMEn between groups differed ($P < 0.05$) at 8, 9, and 10 wk, while CP and P were significantly different from 5 and 6 wk, respectively until 16 wk of age. dLys intake was greater in the HP group from 6 to 11 wk, and cumulative intake of Ca increased ($P < 0.05$) in the LP pullets from 11 up to 18 wk compared to the HP flocks. Optimum cumulative P intake of 14.5, 16.9, 19.4, 22.2, and 25.0 g from 8 to 12 wk, respectively, improved the HHEP at 55 wk. The MLR by wk resulted in significant effects ($P < 0.05$) of growth and nutritional factors at different rearing ages in the HHEP at 55 wk. From 11 to 17 wk, MLR usually included the quadratic effects of CP, Ca, and dLys while accounting for the linear responses of BW, AMEn, and P ($R^2 = 0.82$). In conclusion, BW, FU, and CNI at specific periods during rearing affected the reproductive performance. Data analytics aid in identifying those critical periods using commercial data.

Key Words: Amino acids, Crude protein, Broiler breeders, Rearing

Physiology, Endocrinology and Reproduction: Turkeys

M4 The Turkey Elicits a Dysregulated Immune Response to *Histomonas meleagridis* Infection Katherine Cupo^{*1GS}, Elle Chadwick², Oluwamuyiwa (Gabriel) Akerele³, Robert Beckstead⁴, Ramesh Selvaraj³, Chongxiao (Sean) Chen¹ ¹North Carolina State University, ²Biomim, ³University of Georgia, ⁴Ceva

Histomonas meleagridis is an economically important poultry parasite causing histomoniasis, a disease that results in necrosis of the ceca and liver. Both chickens and turkeys may become infected with the parasite, but turkeys are more likely to developing histomoniasis. Little is understood about the mechanistic interactions between the parasite and either species; information necessary for development of targeted histomoniasis vaccines. Therefore, the goal of this study was to investigate differences between the chicken's and the turkey's immune response to *H. meleagridis* utilizing RNA sequencing (RNA-seq). Broiler breeder chicks and turkey poults were inoculated with 100,000 *H. meleagridis*/bird at 25 days of age. At 5 and 10 days post inoculation (dpi), cecal tonsil and liver tissue samples were collected from 3 infected and 3 non-infected chickens and turkeys for RNA isolation. RNA-seq and differential expression analyses were performed in CLC Genomic Workbench based on a q-value of

0.05. Pathway enrichment analysis was performed in Ingenuity Pathway Analysis software using foldchange cutoffs of -2 and +2 at a q-value of 0.05. At 5 dpi, a total of 6, 200, 1084, and 1817 DEGs were identified in the chicken cecal tonsil, chicken liver, turkey cecal tonsil, and turkey liver, respectively. At 10 dpi, a total of 86, 542, 80, and 2917 DEGs were identified in the chicken cecal tonsil, chicken liver, turkey cecal tonsil, and turkey liver, respectively. The larger number of DEGs identified in the turkey tissues suggests that the turkey has a greater physiological response to the *H. meleagridis* infection than the chicken. Few immune-related pathways were enriched in the chicken cecal tonsil or liver at either time point; however, several immune pathways were enriched in both tissues from the turkey at either time point. Both T_H1 and T_H2 pathways are enriched in the turkey cecal tonsil at 5 dpi and liver at 10 dpi. These pathways are inhibitory of each other and are stimulated in response different types of pathogens. This analysis suggests that the turkey fails to control *H. meleagridis* infection because it elicits a dysregulated immune response leading to hyperinflammation and organ damage.

Key Words: *Histomonas meleagridis*, histomoniasis, RNA-seq, differential expression, immune response

Physiology, Endocrinology and Reproduction: Broilers

M5 Effect of cyclic heat stress on gut barrier integrity, performance and processing traits, and feeding-related hypothalamic neuropeptides of three broiler lines and their ancestor junglefowl Giorgio Brugaletta^{*1,2GS}, Travis Tabler², Elizabeth Greene², Nedra Abdell³, Alison Ramser², Federico Sirri¹, Sara Orlowski², Sami Dridi² ¹Department of Agricultural and Food Sciences, Alma Mater Studiorum – University of Bologna, ²Center of Excellence for Poultry Science, University of Arkansas, ³Animal Nutrition and Welfare Service, Department of Animal and Food Sciences, Universitat Autònoma de Barcelona

Heat stress (HS) endangers the poultry sector sustainability worldwide. Moreover, modern high-performing chickens are more susceptible to HS than their forbears due to higher metabolic rates. HS-caused performance drops are mainly attributed to decreases in feed consumption. As feed intake (FI) is monitored by the hypothalamic centers of hunger and satiety via neuropeptides, this study aimed to determine the effect of cyclic HS on feeding-related hypothalamic neuropeptides (FRHN) of the chicken ancestor junglefowl (JF) and three broiler lines from different periods of genetic selection (i.e., the slow growing ACRB, the moderate growing 95RN, and the fast growing MRB). From 29 to 56 d, birds ($n = 150$ /population) were exposed to either thermoneutrality (TN, 25 °C) or cyclic heat stress (HS, 36 °C, 0900-1800 h). Core body temperature (CBT) and blood serum level of fluorescein isothiocyanate-dextran (FITC-D) as a marker for gut barrier permeability (29 d after HS exposure), performance (0-56 d) and processing (56 d) traits, and FRHN gene expression (56 d) were measured. Data were analyzed through two-way ANOVA with interaction between environmental temperature and line. HS significantly increased CBT and serum FITC-D level while decreased FI and body weight (BW) of MRB and 95RB, but not those of JF and ACRB. The greatest FI and BW were shown by MRB, followed by 95RB, and JF and ACRB under both TN and HS. HS lowered dock and carcass weights in MRB and 95RB, while reduced the overall breast yield and breast pH at 15 min and

4 h postmortem ($p < 0.05$). The expression of major FHRN, like neuropeptide Y, agouti-related peptide, proopiomelanocortin, and cocaine and amphetamine regulated transcript remained unchanged. However, melanocortin receptor 1 expression showed a decreasing trend across lines under both TN and HS ($p = 0.09$), adiponectin expression a trend with 95RB exhibiting the highest mRNA level regardless of the environmental temperature ($p = 0.08$), and JF had greater visfatin mRNA abundance than ACRB under TN ($p < 0.05$). In conclusion, HS potentially caused a leaky gut syndrome in high-performing broiler lines and had greater negative impacts on their performance and processing traits, whereas the expression of FRHN was environmental- and line-dependent.

Key Words: Broiler chicken, Ancestor, Heat stress, Feed intake, Hypothalamic neuropeptides

M6 Effects of dietary bacitracin or *Bacillus subtilis* on the woody breast myopathy-associated gut microbiota of *Eimeria* spp. challenged and unchallenged broilers Linan Jia^{*1GS}, Xue Zhang², Xiaofei Li³, Chuan-Yu Hsu⁴, M. Schilling², Aaron Kiess⁵, E. Peebles¹, Li Zhang¹ ¹Department of Poultry Science, Mississippi State University, ²Department of Food Science, Nutrition and Health Promotion, Mississippi State University, ³Department of Agricultural Economics, Mississippi State University, ⁴Institute for Genomics, Biocomputing, and Biotechnology, Mississippi State University, ⁵Prestage Department of Poultry Science, North Carolina State University

Woody breast (WB) is a meat quality problem that has caused significant economic losses for the broiler industry. A recent study suggested that dysbiosis of the gut microbiota may play a role in the etiology of WB. In the current study, the cecal bacterial communities in chickens fed three different diets were investigated. A 3 (Diet) × 2 (Challenge) factorial arrangement of treatments was used in this study. The experimental diets were a control diet (corn-soybean meal basal diet), an antibiotic diet (basal diet +

6.075 mg bacitracin/kg feed), and a probiotic diet (basal diet + 2.2×10^8 CFU *Bacillus subtilis* PB6/kg feed). On d 14, birds that were assigned to the challenge treatment received a $20 \times$ live cocci vaccine. On d 41, breast muscle hardness in live birds was determined by palpation and grouped into normal (NB) and WB phenotypes. Cecal contents were collected and their bacterial compositions were analyzed and compared. The genomic DNA of the cecal contents was extracted and the V3 and V4 regions of *16S rRNA* gene were amplified and sequenced via an Illumina MiSeq platform. There were no differences ($P > 0.05$) in Shannon and Chao 1 indexes between the 2 challenges, 3 diets, and 2 phenotypes (NB vs. WB). However, there was a difference ($P = 0.001$) in the beta diversity of the samples between the challenged and non-challenged groups. Relative bacterial abundance differed (false discovery rate, $FDR < 0.05$) between the challenge treatments, but there were no significant differences ($FDR > 0.05$) among the three diets or two phenotypes. Predicted metabolic activities only differed ($q\text{-value} < 0.05$) between challenged and non-challenged groups. The cocci challenge altered the gut microbial composition, but the dietary antibiotic and probiotic treatments did not impact gut microbial composition. No strong association was found between WB myopathy and gut microbial composition, which is contrary to the hypothesis that the gut microbiota may contribute to the development of WB. Future research should focus on various gut health conditions, such as oxidative stress and gut barrier function which may explain why the dietary additives affected WB incidence in other research.

Key Words: antibiotic, probiotic, coccidiosis, breast muscle myopathy, gut microbiota

M7 Effects of antibiotic growth promoters on amino acid partitioning and intestinal physiology in commercial broilers Charles Meeks^{GS}, Laura Ellestad *University of Georgia*

Subtherapeutic levels of antibiotics have historically been used in the broiler industry for growth promotion. Implementation of the 2017 Veterinary Feed Directive has barred this practice, generating a need to establish antibiotic growth promoter (AGP) alternatives. However, the mechanism by which AGPs influence broiler growth efficiency remains unknown. The objective of this study was to investigate the effects of AGPs on intestinal physiology and nutrient utilization by assessing amino acid (AA) levels in plasma and intestinal mucosa and AA transporter gene expression in several tissues. Male broiler chicks ($n=216$) were individually caged in an environmentally controlled room between day 10 (d10) and d36 and received one of two grower (d10-d24) and finisher (d24-d36) diets: an antibiotic-free control diet or a diet containing subtherapeutic levels of bacitracin methylene disalicylate (55 mg/kg diet). Birds and feed were weighed to determine individual feed conversion ratio (FCR). On d36, birds with lowest FCR (high efficiency; HE) or highest FCR (low efficiency; LE) from each diet were sampled for liver tissue and jejunum mucosa to measure gene expression ($n=8$) and AA analysis in mucosa ($n=4$). Also, plasma was collected on d21, d28, and d36 ($n=4$). Gene expression was determined using reverse-transcription quantitative PCR (RT-qPCR). AA levels were determined by LC-MS/MS. Data were analyzed by ANOVA

and *post hoc* means comparisons were made with the test of least significant difference when ANOVA indicated statistical significance ($P \leq 0.1$). Arg, Glu, and His were significantly lower in plasma of AGP-fed birds at d36, with a similar trend at earlier ages, and Tyr in jejunum mucosa was significantly higher in AGP fed birds. In jejunum mucosa, cationic AA transporter (*CAT*) 1 & 2, excitatory AA transporter 3, and alanine/serine/cysteine/threonine transporter 1 were all found to be decreased in expression in LE AGP-fed birds compared to LE control-fed birds. In liver, *CAT1* and Y+L AA transporter 2 were found to have decreased expression in HE AGP-fed birds compared to HE control-fed birds. In conclusion, these results suggest AGPs could affect growth efficiency in broilers, in part, by affecting uptake and partitioning of AAs, thus affecting bird physiology.

Key Words: antibiotic growth promoters, amino acids, intestine, broiler, feed efficiency

M8 The effects of triiodothyronine in avian muscle cells may be regulated through the insulin-like growth factor binding proteins Lauren Vaccaro^{GS}, Addison Smith, Kyle Herring, Laura Ellestad *University of Georgia*

The thyrotropic and somatotrophic axes engage in crosstalk in mammalian systems while regulating growth and metabolism. This includes modulation of the insulin-like growth factors (IGFs) and IGF binding proteins (IGFBPs) by the thyroid hormones (THs). Previously, we found that several IGFBPs were differentially expressed in the breast muscle of modern and legacy broilers and identified potential TH response elements in select IGFBP promoter regions. This suggests that the IGFBPs contribute to the physiology of modern broilers and their actions may be controlled, in part, by TH signaling. This study's objective was to determine effects of triiodothyronine (T_3) on somatotrophic and thyrotrophic gene expression in Quail Muscle Clone 7 (QM7) satellite cells. Undifferentiated QM7s were treated with 0, 1, 5, or 25 ng/mL T_3 for 0.5, 6, or 24 hours and mRNA expression was determined by RT-qPCR ($n=4$). Data were analyzed by two-way ANOVA, and *post hoc* means comparisons were made with the test of least significant difference when ANOVA indicated significance ($P \leq 0.05$). Treatment with 1 ng/mL T_3 increased *IGFBP2* expression ($P \leq 0.05$), but this was not observed when 5 or 25 ng/mL were administered T_3 . *IGFBP3* expression was elevated and approached significance with 1 ng/mL T_3 ($P=0.0649$). *IGFBP5* expression increased with all T_3 treatments ($P \leq 0.05$). By 24 hours, cells treated with 1 ng/mL T_3 had higher levels of *GHR* than those with treated with 5 or 25 ng/mL T_3 ($P \leq 0.05$). Thyroid hormone receptor beta (*THRB*) levels peaked at 25 ng/mL ($P \leq 0.05$), while all concentrations significantly increased deiodinase 3 (*DIO3*) expression ($P \leq 0.05$). These data suggest that T_3 can stimulate components of the somatotrophic axis that may contribute to muscle cell proliferation *in vitro*. Specifically, T_3 -induced IGFBP activity could restrict or induce local muscle tissue growth. This could occur through the action of IGFBPs 2, 3, and 5, likely through managing cell proliferation or altering paracrine IGF signaling that could be stimulated by increased GHR activation.

Key Words: thyroid hormone, avian muscle, IGF binding proteins

Physiology, Endocrinology and Reproduction: Laying Hens

M9 Circadian expression of calcification-related genes in the eggshell gland of commercial laying hens Micaela Sinclair-Black^{GS}, Lyssa Blair, Laura Ellestad *Department of Poultry Science, University of Georgia*

Complete eggshell mineralization is critical for improving shell quality and, therefore, salable eggs. Understanding dynamics of gene expression involved in mineralization could improve genetic selection and nutritional strategies geared towards optimizing eggshell calcification. This research aimed to elucidate fluctuations in mRNA expression of genes involved

in transport and utilization of calcium (Ca^{2+}) and bicarbonate (HCO_3^-) in the shell gland (SG) of laying hens over a 24-hour period. Tissue was collected from 32-week old Hyline hens ($n=6$ per time point) at 1, 3, 4, 6, 7, 8, 12, 15, 18, 21, 23, and 24 hours post oviposition (HPOP). Levels of mRNA were determined using real-time quantitative PCR. Data were analyzed by one-way ANOVA, and means were compared using Fisher's Least Significant Difference test when ANOVA indicated significance ($P \leq 0.05$). Sodium dependent Ca transporter *SLC8A1* expression increased gradually from 1 HPOP until it peaked at 12 HPOP ($P \leq 0.05$), then it decreased to levels similar to those at 1 and 3 HPOP ($P \leq 0.05$).

Similarly, other Ca transporters such as *ATP2B1* also peaked around 12 HPOP ($P \leq 0.05$). Calbindin 1, a Ca chaperone located within uterine glandular cells, had low expression during periods without an egg in the shell gland (1 to 4 HPOP) but increased four-fold from 8-12 HPOP ($P < 0.01$). These increases in gene expression coincided with increased calcification; thereafter, they decreased gradually until 24 HPOP. Following a similar pattern, the HCO_3^- transporter *SLC26A9* increased from 1 to 12 HPOP ($P < 0.01$) and decreased at a similar rate from 15 to 24 HPOP, with the lowest expression occurring from 23 to 24 HPOP ($P \leq 0.05$). Carbonic Anhydrase enzyme (*CA2*) forms HCO_3^- from CO_2 and H_2O within uterine glandular cells. The initial expression peak of *CA2* precedes that of the Ca^{2+} and HCO_3^- transporters, with its highest expression plateauing from 6 to 12 HPOP; however, it follows a similar decrease from 12 to 24 HPOP ($P \leq 0.05$). These results suggest that laying hens have the greatest capacity to transport and utilize Ca^{2+} and HCO_3^- in the SG around 12 HPOP. During this period, the hen will require sufficient dietary Ca to decrease bone resorption and support high-quality eggshell production.

Key Words: calcium, layer, egg calcification, shell gland

M10 Effects of the in ovo injection of an *Escherichia coli* vaccine on the hatchability and characteristics of commercial layer chicks L. Lindsey^{1GS}, J. Evans², K. Elliott², S. Fatemi¹, A. Mousstaaid¹, P. Gerard³, E. Peebles¹ ¹*Department of Poultry Science, Mississippi State University*, ²*USDA-ARS, Poultry Research Unit*, ³*School of Mathematical and Statistical Sciences, Clemson University*

Avian pathogenic *Escherichia coli* infections in layers can lead to significant economic losses to the commercial table egg industry. Currently, the

leading recommended vaccine against avian pathogenic *E. coli* in poultry is the Poulvac® *E. coli* vaccine (Zoetis, Parsippany, NJ). The *in ovo* administration of this vaccine in layer hatching eggs has not been previously tested. The purpose of this experiment was to examine the effects of the *in ovo* administration of different doses of the Poulvac® *E. coli* vaccine in fertile Hy-Line W-36 layer hatching eggs on subsequent hatchability and hatchling characteristics. Treatments included non-injected and diluent-injected controls, and the injection of diluent containing a full dose, or 1×10^{-2} , 1×10^{-4} , 1×10^{-6} dilutions of the full dose of the vaccine. Injections (50 μL) targeted the amnion on day 18 of incubation. A full dose was calculated to contain 4.40×10^7 CFU of *E. coli* bacteria. At hatch, percentage hatchability of live embryonated eggs (HE), percentage residue eggs, chick length, whole and yolk-free body weights, and yolk sac weight were examined. Treatment significantly ($P < 0.0001$) affected HE. The highest HE values were observed in the non-injected (97.3%) and diluent-injected control (94.2%) groups, whereas HE was 89.0, 88.9, 84.4, and 71.2% in the 10^{-6} , 10^{-4} , 10^{-2} , and full dose treatments, respectively. The full dose treatment resulted in the highest number of chicks that were alive but remained in the shell at hatch, including internal and external pips. In comparison to all other treatment groups, chick length was significantly ($P < 0.0001$) higher in the non-injected and diluent-injected controls, as well as the 10^{-6} dilution treatment group. Yolk-free chick body weight was significantly lower than controls in the 10^{-2} and full dose treatments ($P = 0.0338$). Layer chicken embryos were successfully *in ovo*-vaccinated with the Poulvac® *E. coli* vaccine at 18 days of incubation. However, hatch success, chick length, and yolk-free body weight decreased with an increase in vaccine concentration.

Key Words: *E. coli*, layers, in ovo, percent hatch, vaccine

Teaching, Pedagogy, Extension

M12 Assessment of retention trends in the college of agriculture and life sciences to determine possible internal recruitment efforts for poultry science departments Peyton Taylor^{GS}, Ryan Walker, Kelley Wamsley, Jessica Wells *Mississippi State University*

Recruitment in poultry science departments is necessary to aid in meeting industry demand for qualified employees. Research from our lab utilized data mining techniques to assess lowest retention rates in departments within colleges of agriculture at the 6 universities with poultry science departments. Data identified Animal Science (AS) and Biochemistry (BCH) to have the lowest retention rates, suggesting a potential target for internal recruitment practices. Therefore, the objective of this study is to determine if non poultry science majors from these universities would be receptive to completing poultry coursework. Undergraduates from these previously determined departments were surveyed ($n=582$) to assess if students would take an introductory poultry course, what factors influenced their current major choice, and if they would be interested in majoring in poultry science. Additionally, faculty members within the same departments were also surveyed ($n=110$) to assess if they would be willing to advise students who are changing their major to consider poultry, and would they allow poultry guest lecturers in their class to expose students. Data collected in the student survey showed a total of 132 students changed their major, with over half of those students changing majors coming from AS and BCH ($n=27$ and 46 , respectively). Thus, solidifying previous data that AS and BCH had the largest population of students change majors. 80% of AS and BCH students indicated an interest in taking a poultry course if it counted towards their curriculum. 31% of students indicated consideration to major in poultry science, after being informed through the survey of opportunities within poultry science. However, 77% of faculty answered "no" to allowing a poultry course into their curriculum as a social science for students, a stark contrast to the student survey and what students would like. This data suggests that there is a disconnect between students and faculty in reference to poultry science curriculum. For future research,

data needs to focus on how to aid students in obtaining information about poultry science as well as changing perceptions of faculty in other departments in relation to the need for poultry curriculum.

Key Words: Recruitment, poultry science, retention rates

M13 Assessment of current hatch-out program curriculum implemented in K-12 for Mississippi Marissa Powell^{GS}, Ryan Walker, Kelley Wamsley, Jessica Wells *Mississippi State University*

A lack of agriculture education in the classroom could negatively impact the agriculture industry, meaning implementing agricultural literacy in classrooms is imperative. Mississippi State University has utilized a program (Hatch-out Program) to incorporate poultry curriculum into K-12 classrooms. Previous survey data from our lab concluded that the current curriculum needed minor modifications. Therefore, the objective of this study is to implement the revised curriculum into K-2 classrooms across Mississippi utilizing a pre- and post- test to determine outcomes, and a post- survey to participants to evaluate the curriculum. Utilizing 9 schools, the revised program was implemented in 71 classrooms, reaching 1,471 students. Following implementation of this revised curriculum, there was a 68% average increase in knowledge for participants, with each grade (K-2) showing an individual percent increase of 69%, 67%, and 69% respectively. Additionally, in the pre- and post-exam, a qualitative drawing assessment was also utilized, and results demonstrated an increase in detail of poultry farms in all three grades. Thus, further strengthening data illustrating knowledge gained from the curriculum instruction. The post survey was used to determine satisfaction with the program, ease of implementation, and age- appropriateness in regard to the lesson (PowerPoint), curriculum, teacher narrative, and activities provided. With a 70% return rate ($n=45$), post surveys of participants indicated that 100% of teachers were satisfied with the program. 87% of teachers reported an ease of implementation, with 32% providing comments in relation to time constraints

being problematic with implementation. 70% of participants recorded a “yes” when asked if the pre- and post- test was age appropriate. Out of the 30% who responded “no”, 69% were K level teachers. This, coupled with other survey details led to a conclusion that the curriculum may need more modification for K level but proves to be sufficient for grades 1-2. Future research needs to focus on meeting time constraint needs of teachers as well as modify curriculum for kindergarten.

Key Words: agricultural literacy, curriculum, k-12, hatch-out program, poultry

M14 The development, implementation, and evaluation of the Hybrid-Online Hatch-Out Program Tannah Christensen^{*1GS}, Donna Peterson¹, Jessica Wells², Mariah Morgan¹, Marina Denny¹ ¹*School of Human Sciences, Mississippi State University, 2**Department of Poultry Science, Mississippi State University*

Today's society lacks knowledge about agriculture, leading to a demand for agricultural programs to increase agricultural literacy. Mississippi State University Poultry Science Department offers a program providing elementary students in the state an experiential poultry learning curriculum. There are 82 counties in MS and limited staff to implement the current hatch-out program using a face-to-face delivery method. Therefore, the proposed objective of this study was to develop an online version to reach a larger population within the state. This study aimed to develop and evaluate the Hybrid-Online Hatch-out Program. Outcome evaluation was conducted to assess knowledge change among participants, while pro-

cess evaluation was used to document satisfaction and implementation. A one-group pretest-posttest design was used to assess outcomes, while a post-only design was used to assess process. The program was pilot tested in two elementary schools with 26 teachers and 529 students (4K-1st). A total of 125 students completed both pre and post-tests and 16 teachers completed at least 1 of the 8 surveys. There was a significant increase in the overall scores of students from pre- to post-test ($p=0.00001$). Teachers were asked to complete a Teacher Pre-Survey, 6 Module Surveys, and a Teacher Post-Survey to assess process evaluation components as well as teacher knowledge gained from the program. Overall, teachers were 100% satisfied with the program. On average, teachers ($n=16$) indicated having a high comfort level when using an online platform, and those that answered ($n=7$) had no issues with technology in the online portion of the curriculum. Results indicated teachers had better knowledge of both agriculture and poultry after program implementation. Prior to program implementation only 19% of teachers ($n=16$) indicated having a good level of knowledge of agriculture, while after program implementation 72% of teachers ($n=7$) indicated having a good knowledge level in agriculture. Furthermore, 100% of teachers ($n=7$) indicated they were likely to implement the program again. This data concluded that an online option has the potential for aiding in reaching more participants to increase ag literacy (specifically poultry) in the state of MS.

Key Words: agricultural literacy, agricultural education, hybrid-online, curriculum, hatch-out program

Processing and Products

M15 Effects of Syngenta Enogen® corn on broiler breast objective and subjective color and stability measurements Morgan Gravely^{*1GS}, Oscar Martinez², Hanna Alcocer¹, Xiaoxing Xu¹, Zhijun Wang¹, Alexander Stelzleni¹, Harshavardhan Thippareddi², Woo Kim², John Gonzalez¹ ¹*Department of Animal and Dairy Science, University of Georgia, 2**Department of Poultry Science, University of Georgia*

The objective of this study was to examine Syngenta Enogen® corn effects on broiler breast color and stability during simulated retail display. Day-old chicks ($N=720$) Cobb 500 males were randomly allocated to 36 floor pens that had feeders and waters that allowed *ad libitum* access to both. Each pen was randomly assigned 1 of 4 treatments: (PC): conventional poultry diet containing corn and soybean meal, (NC): PC –200 kcal/kg ME, (NCS): NC replaced with Syngenta Enogen corn, and (NCE): NC +80 kilo-Novo α -amylase units/kg. On experiment day 43, birds were weighed and five mean-weight birds per pen were selected for harvest. Birds were harvested under simulated industry conditions, carcasses chilled in ice-water baths for 3 hours, breasts were placed on styrofoam trays with absorbent pads and overwrapped with polyvinylchloride film, and displayed in coffin-style retail cases for 7 days. Objective and subjective color measurements were collected by spectrophotometer and visual panel, respectively, and data were analyzed as a completely randomized design with repeated measures. There were no Treatment \times Day interactions or Treatment effects for L* (lightness) and a* (redness) values ($P>0.11$). There were Treatment \times Day interactions ($P<0.01$) for b* (yellowness) and chroma (vividness). From day 0 to day 7, chroma values increased by 4.3%, 2.9% and 2.5% for PC, NC and NCE, respectively, while NCS remained essentially unchanged (-0.4%). Similarly, b* values increased by 7.4%, 5.1% and 5.0% for PC, NC and NCE, respectively, over 7 days while NCS increased by only 0.5%. There were no Treatment \times Day or Treatment effect for visual panel lean color ratings ($P>0.15$). There was an interaction ($P=0.01$) for discoloration scores, where there were no treatment differences through display day 3 ($P>0.28$), but from day 4 to 7 NCS breasts had between 2.5% and 5.7% less discoloration. Utilizing Enogen corn in broiler diets decreased objective breast yellowness and vividness,

but differences were not seen by visual panelists. These objective color differences did manifest as improvements in Enogen breast color stability at the end of simulated retail display.

Key Words: broiler, color, retail display, spectrophotometer, visual panel

M16 Analyzing Salmonella serovar dynamics using selective pre-enrichment conditions Surendra Rasamsetti^{*1GS}, Nikki Shariat¹, Mark Berrang², Nelson A. Cox² ¹*Department of Population Health, Poultry Diagnostic and Research Center, University of Georgia, 2**USDA-Agricultural Research Service, U.S. National Poultry Research Center*

Conventional *Salmonella* surveillance requires a week for isolation, confirmation, and subsequent serotyping. We previously showed that this time burden could be reduced by 24 hours by combining the pre-enrichment and enrichment steps. We added selective components from the traditional *Salmonella* enrichment broths to BPW after four hours of a non-selective pre-enrichment step. The selective pre-enrichment culture resulted in *Salmonella* recovery from 89% (16/18) samples when plated directly after selective pre-enrichment and the serovar profile analyzed was comparable to traditional culture conditions. This work was performed on broiler carcasses directly after picking, where we expected the *Salmonella* load to be high. Here we sought to investigate the efficacy of selective pre-enrichment to isolate *Salmonella* at five different points during processing from high to low *Salmonella* incidence. Duplicate samples, each representative of 500 broiler carcasses, were collected by catching drips under moving carcass shackle lines at post-pick, post-IOBW, post-chill plus pre- and post-dip for wings over six different collections ($n=60$) from a processing facility. Carcass drip samples were cultured under selective pre-enrichment conditions and in parallel with BPW pre-enrichment followed by RV and TT selective enrichment. The number of positives identified with selective pre-enrichment conditions (29/60) was similar to traditional selective enrichment culture conditions (27/60) (Fisher's exact test, $P=0.8549$). Importantly, the incidence of *Salmonella* reduced dramatically after antimicrobial intervention (post pick 75% vs. post chill 17%). Col-

lectively, these results suggest that a selective pre-enrichment step reduces the time required for *Salmonella* isolation.

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

M17 Quantification of *Campylobacter jejuni*, *coli* and *lari* in poultry post-chill whole bird carcass rinses utilizing a shortened enrichment time and PCR Aaron Bodie^{*1GS}, Dana Dittoe¹, Savannah Applegate², Tyler Stephens², Steven Ricke¹ ¹*University of Wisconsin*, ²*Hygiene*

Campylobacter is one of the leading causes of gastroenteritis in the United States, with poultry being a major reservoir. Currently, the culture plate method using Campy-Cefex agar is the gold standard for the quantification of *Campylobacter* spp. among poultry products. However, this methodology is not sensitive enough to quantify the viable but non culturable cells leading to false negatives. Therefore, a novel method for the detection and quantification of *Campylobacter* spp. is needed to improve food safety among consumers. The objective was to develop and optimize a rapid quantification method for *Campylobacter* species (CampyQuantTM) in post-chill poultry rinsates using the BAX[®] System Real-Time PCR Assay for *Campylobacter* (RT-Campy). Per serovar, *C. jejuni* ATCC 700819, *C. coli* ATCC BAA-1061, or *C. lari* ATCC BAA-1060, 30 mL of bulk post-chill whole bird carcass rinsates were aliquoted to 24 oz Whirl-Pak bags (N=39). Samples were either uninoculated (n=1) or inoculated with 1 to 4 Log₁₀ CFU/mL of (n = 3) of *Campylobacter* spp. Pre-warmed (42°C) 2' Blood free Bolton's Broth (30 mL) with 2' antibiotic supplement was added to each sample and enumerated on Campy-Cefex. Samples were incubated at 42°C for 20 h and tested in quintuple with the BAX[®] Q7 RT-PCR system. The results were then compared using a Chi-Square test to estimate the initial inoculation level of each *Campylobacter* species on Campy-Cefex in relation to the initial inoculation levels ($P < 0.05$). A linear fit equation was generated for each *Campylobacter* species using the cycle threshold (CT) from the BAX[®] System to estimate pre-enrichment Log₁₀ CFU/mL of rinsates. The statistical parameters of each equation yielded a R² of 0.93, 0.76, and 0.94 with a Log₁₀ RMSE of 0.28, 0.57, and 0.27 from *C. jejuni*, *C. coli*, and *C. lari*, respectfully. Campy-Cefex and CampyQuantTM estimations were not statically different. The study suggests that the BAX[®] System Real-Time PCR Assay for *Campylobacter* can provide the food industry with a rapid, accurate, and efficient alternative method for *Campylobacter* enumeration in poultry processing rinses.

Key Words: *C. jejuni*, *C. lari*, *C. coli*, PCR, Quantification

M18 Reduction in *Campylobacter jejuni* colonization in the ceca by coarse spray of an inactivated vaccine in broilers. Charles Hofacre^{*1}, Roy Berghaus², Bereket Zekarias³, Sam Christenberry³, Hector Cervantes³, Luis Gomez³, Peter Winter³, Karen Brown³, Matthew Jones¹, Jennie Baxter¹ ¹*Southern Poultry Research Group, Inc.*, ²*The University of Georgia*, ³*Phibro Animal Health*

Campylobacter jejuni is the most common cause of bacterial foodborne illness according to the CDC. *C. jejuni* (C.J.) readily colonizes broiler chickens' ceca and can be shed in feces at very high numbers. If these numbers in the broilers' intestines can be reduced before slaughter, the processing plant may be able to reduce the number of *C. jejuni* on broiler carcasses thus reducing the risk of human *Campylobacter* illness. This study evaluated an inactivated vaccine of *C. jejuni* J.B. strain with three adjuvants (carbigen, a commercial adjuvant and two experimental polymer based adjuvants, 222 and 111). These were administered to 1600 Ross 708 broilers by coarse spray (0.25 ml/chick) on days of age 1 and 7. There were eight replicates: challenge control, adjuvant carbigen, adjuvant 111 and adjuvant 222. On day 14 the homologous *C. jejuni* JB strain was orally gavaged to 100% of the birds at 3.1×10^5 CFU/chick. On days 35 (10 birds) and days 42/43 (15 birds) were euthanized and ceca removed. Each ceca had MRD broth added and 10-fold dilutions in 96 well blocks plating 0.1 ml from 10⁴ to 10⁸ onto Campy Cefex Agar. All of the pens were C.J.

bootsock positive at 21 and 42 days indicating a uniform challenge. There was not a significant reduction in C.J. ceca prevalence on day 35 or 42/43; however, on day 35, adjuvant 222 had 43.8% prevalence while the challenge control was 62.5%. Enumeration of the 35-day culture-positive ceca demonstrated adjuvant 222 had a similar trend at 5.47^a log₁₀ CFU/g and challenge control 6.17^{ab} log₁₀ CFU/g. Since no culture method can detect extremely low numbers, a CFU = 0 was censored to 3.0 log₁₀ CFU/g. A Tobit regression analysis of this data demonstrated the 35day C.J. ceca numbers for adjuvant 222 was significantly reduced at 3.31^a log₁₀ CFU/g versus control at 4.60^b log₁₀ CFU/g. There was no significant effect by any treatment observed in the 42-day ceca samples in prevalence or number. Results of this study indicated that a coarse spray applied autogenous *Campylobacter jejuni* bacterin with adjuvant 222 can reduce campy colonization in broilers early in the infection. Future studies will be needed to determine the ideal vaccination boost time to successfully lower *Campylobacter* at different processing ages.

Key Words: *Campylobacter*, Food Safety, Inactivated Vaccine, Broilers

M19 Isolation and characterization of *Campylobacter jejuni* from 'no antibiotics ever' raised broilers Sabin Poudeh^{*1GS}, Anuraj Sukumaran¹, Pratima Adhikari¹, Aaron Kiess², Li Zhang¹ ¹*Department of Poultry Science, Mississippi State University*, ²*Prestage Department of Poultry Science, North Carolina State University*

Campylobacter jejuni (*C. jejuni*) is a leading bacteria causing food-borne illness, and poultry is considered as its major reservoir host. Production of broilers under 'no antibiotics ever' (NAE) system has increased in recent years. In 2020, NAE-raised broilers accounted for ~50% of the total broilers produced in the USA. However, the impact of this shift to NAE on the prevalence and characteristics of *C. jejuni* has not been studied. The objective of this study was to evaluate the prevalence and virulence characteristics of *C. jejuni* in NAE-raised broilers. Cloacal swabs were collected from the live-hang areas of three commercial processing plants over a 9-week period. Each plant was visited three times with an interval of one week and per visit, 30 samples were collected. *C. jejuni* was isolated following the Microbiology Laboratory Guidebook (MLG) 41.04, and presumptive isolates were confirmed via PCR using the *C. jejuni* specific *hippo* gene. The isolated *C. jejuni* were tested for virulence genes and genotypic and phenotypic antibiotic resistance. Out of the 270 samples, 44 (16.3%) samples were *C. jejuni* positive. The detected virulence genes included (prevalence in parenthesis) adhesion genes [*cadF* (65.9%), *jlpA* (52.3%), *pebA* (36.4%), *pldA* (81.8%)], motility factor genes [*flaAB* (79.5%), *flgB* (81.8%), *flhB* (63.6%)], the invasion gene *ciaB* (65.9%), and toxin genes [*cdtA*, *cdtB*, *cdtC* (65.9%)]. However, type IV secretion gene *virB9* and adhesion gene *porA* were not detected. The prevalence's of resistance genes for quinolones (*gyrA*), aminoglycosides [*aph* (3')-IIIa], and macrolides [*erm*(B)] were 100%, 59.1%, and 50%, respectively. Out of the 41 *C. jejuni* evaluated for antibiotic susceptibility, eight isolates (19.5%) exhibited multi-drug resistance, and the highest resistance was observed against tetracycline (31.7%), whereas 24.4% of the isolates were resistant to clinically important antibiotics (azithromycin and ciprofloxacin). The presence of multi-drug resistance and virulence genes among *C. jejuni* isolates from NAE-raised broilers indicate a potential for serious human illnesses if transmitted via the food chain. This study provides important insights into the prevalence, virulence properties, and antimicrobial resistance of *C. jejuni* in NAE-raised broilers.

Key Words: *Campylobacter jejuni*, food safety, antibiotics resistance, virulence gene, NAE-raised broiler

M20 Effect of exsanguination method on rate of blood loss and total blood loss in broilers Rachel Osborne^{*1GS}, Emily Baethke¹, Caitlin Harris^{1,2}, Richard Buhr², Brian Kiepper¹ ¹*Department of Poultry Science, University of Georgia*, ²*Poultry Microbiological Safety and Processing Research Unit, U.S. National Poultry Research Center; Richard B. Russell Agricultural Research Center, USDA-ARS*

Although blood represents a relatively low proportion of a broiler's total body composition, with over 9 billion broilers processed each year in the U.S., blood is not an insignificant by-product of poultry processing. Yet, there is relatively little research into the effect of exsanguination method on overall blood loss and blood loss rate. It has been theorized that decapitation may result in a less effective bleed out than a conventional neck cut. However, there are no definitive studies examining this issue with modern-day, feed withdrawn broilers such as could be found in a commercial processing environment. Without access to accurate bleed out data, processors may find themselves having to arbitrarily decide on an appropriate bleed out time and blood collection tunnel length. The aim of this study was to determine percent of blood loss and rate of blood loss for multiple exsanguination methods. To accomplish this, 88 male broilers at 51d of age underwent an 8-hour feed withdrawal before obtaining a live weight (mean = 3896 g). Birds were then electrically stunned and randomly assigned to be exsanguinated in one of 4 ways (n = 22): neck cut on one side (1S), neck cut on both sides (2S), decapitated at the base of the head (DH), or decapitated at the base of the neck (DN). Post exsanguination, bird weights were recorded in 15s intervals until 3 minutes had elapsed. Rate of blood loss and percent total blood loss were calculated, and data were analyzed by ANOVA in SAS JMP using Student's t-test for means separation. Over the 3 minutes that blood loss was measured, DN (2.69%) showed a significantly lower cumulative %blood loss than 2S (2.99%), DH (3.01%), and 1S (3.01%) ($P=0.0286$). For the 0-15s post-neck cut period ($P<0.0001$), the DH (1.88%) and DN (1.64%) treatments were not significantly different from each other, but the DH treatment was significantly greater than the 2S (1.55%) and 1S (1.25%) treatments. After the first 15s period, the 1S treatment had a significantly higher blood loss rate than the other treatments for the 30s, 45s, 60s and 75s periods. No significant differences were seen between treatments after 75s. These findings provide insight into how best blood collection and bleed out time may be optimized when processing the modern broiler.

Key Words: poultry processing, poultry by-products, blood loss, exsanguination, bleed out time

M21 Meat quality of broiler chickens processed using electrical and controlled atmosphere stunning systems Montana Riggs^{*GS}, Rüdiger Hauck, Bethany Baker-Cook, Rachel Osborne, Amrit Pal, Maria Bethonico Terra, Gracie Sims, Andrea Urrutia, Leticia Orellana Galindo, Marco Reina, Dianna Bourassa *Auburn University*

Increased consumer concern for animal welfare has led some poultry producers to alter their stunning methods from electrical to controlled atmosphere stunning. The impact on meat quality between controlled atmosphere stunning (CAS) and electrical stunning (ES) using current U.S. parameters has not been evaluated. To assess the impact of each stunning method on meat quality, three trials were conducted in a commercial broiler processing facility. Blood glucose concentrations were measured from broilers stunned by either CAS or ES at the following stages: 1) Lairage 2) Pre-stun and 3) Post-stun, using a glucose monitor. Visible wing damage was evaluated post-defeathering and breast fillet meat quality was evaluated through measurement of pH, CIE-LAB values, and drip loss. Values were determined both at deboning and 24 hours after deboning. Data were analyzed by GLM or Chi-Square through SAS with a significance at $P\leq 0.05$ and means were separated by Tukey's HSD. Blood glucose concentrations (mg/dL) from CAS and ES birds were not different at lairage (284, 272, $P=0.2646$) or immediately prior to stun (274, 283, $P=0.6425$). Following stunning and neck cut, CAS blood glucose was significantly higher than ES blood glucose (418, 259, $P<0.0001$). CAS carcasses

had significantly more visible wing damage than ES carcasses (3.52%, 2.22%, $P<0.0001$). Breast fillet pH, L*, and a* values from CAS (5.81, 54.65, 1.96) differed from ES (5.92, 53.15, 2.31, $P<0.0001$, $P=0.0005$, $P=0.0303$). There was no difference found when evaluating b* values at debone ($P=0.7162$). No significant differences were detected for b* at deboning or for pH, CIE-LAB values, or drip loss at 24 hours post-debone ($P\geq 0.0859$). The implications of increased blood glucose concentration post-CAS are currently unknown and will require further evaluation. Although differences were observed in breast fillet attributes at deboning, the degree of difference would have minimal practical application and were no longer present at 24 hours. However, the increase in visible wing damage observed post-pick from CAS carcasses may be a concern when using CAS for broiler stunning.

Key Words: broiler, stunning, meat quality, glucose

M22 Textural characteristics of raw pet treats developed from broiler chicken wing tips Gerardo A. Abascal-Ponciano^{*1GS}, Jorge Sandoval¹, John Rogers¹, Jorge Romero¹, Justin Dunavant¹, Marc Presume¹, Said Herrera¹, Josh Renew¹, Jorge Banegas¹, Cristopher Almendares¹, Diego Ventura¹, Luis Guzman¹, Tristan Reyes², Madison Wagoner², Robert Mason³, Eric Altom³, Jason Sawyer², Jessica Starkey¹, Charles Starkey¹ ¹*Auburn University Department of Poultry Science*, ²*Auburn University Department of Animal Science*, ³*Balchem Corporation*

Chicken wing tips (WT) are low-value co-products generated from broiler chicken processing. Transforming WT into pet treats could improve the value of these processing co-products. Structure forming technologies such as sodium alginate (SL) and encapsulated calcium lactate (ECL; ALGIN) have the potential to aid in the formation of pet treats from co-products such as WT. The objective of this study was to assess the effect of different ALGIN inclusions on textural characteristics of raw WT-derived pet treats. Previously frozen WT were ground with a 4.8-mm grinder plate and mixed with 1 of 4 concentrations of ALGIN: 0.5x (0.50% SL + 0.425% ECL); 1x (1.00% SL + 0.85% ECL), and 2x (2.00% SL + 1.70% ECL) the manufacturer's recommended inclusion rates plus a control group with no ALGIN inclusion (0x). After mixing, each treatment was extruded into 63.5-mm diameter casings, stored at -20°C for 24 h, and sliced into 5-mm thick slices. A TA-HDplusC texture analyzer was used to evaluate (n = 10 samples from each treatment per analysis). A TA-42 probe was used for the three-point bend test to assess: stiffness (gradient of force to distance), hardness (force used to compress), and flexibility (distance of compression), while a TA-43R probe was used for the shear force test to assess toughness (total force over distance) and firmness (peak force). Data were analyzed as a 1-way ANOVA using the GLIMMIX procedure of SAS (v9.4) and least square means were separated with the PDIF option at $P\leq 0.05$. Tendencies were declared at $0.0501\leq P\leq 0.1$. Firmness ($P=0.7947$) and stiffness ($P=0.6637$) of raw pet treats were not influenced by ALGIN inclusion. Pet treats with 1x and 2x ALGIN inclusions were tougher and more flexible compared with treats containing 0x and 0.5x ALGIN ($P<0.0001$). Raw treats produced with 2x ALGIN tended to be harder compared with those containing only 0x and 0.5x ALGIN but were similar to those with 1x ALGIN ($P=0.0712$). In conclusion, increasing ALGIN inclusion did not impact stiffness or firmness of WT-derived raw pet treats but increased their hardness, flexibility, and toughness. Addition of at least 1x ALGIN was required to impact the shear force and 3-point-bend characteristics of raw WT-derived pet treats.

Key Words: alginate, calcium lactate, chicken wing tips, pet treats, textural characteristics

M23 Evaluation of textural characteristics of raw pet treats generated from mixtures of broiler liver and heart

Jorge Banegas^{*1GS}, Marc Presume¹, Rigo Soler¹, Jorge Sandoval¹, Justin Dunavant¹, Jorge Romero¹, Luis Avila¹, Moses Chilenje¹, Catherine Odom¹, Laura Garner¹, Amit Morey¹, Robert Mason², Eric Altom², Charles Starkey¹ ¹*Department of Poultry Science, Auburn University*, ²*Balchem Animal Nutrition and Health, Balchem Corp.*

Broiler chicken processing generates low value co-products such as livers and hearts that have potential for inclusion in pet treats as sources of protein and flavor. Textural characteristics can influence quality and buyer decisions. Hydrocolloid stabilizing agents such as ALGIN (sodium alginate (SA) and encapsulated calcium lactate (ECL)) can be included in ground meats to improve texture. The objective was to evaluate the textural characteristics of pet treats generated from mixtures of raw CL and CH containing 1 of 2 ALGIN concentrations. Chicken livers and CH were ground with a 4.8-mm grinder plate and mixed in 3 different ratios: 25% CL:75% CH, 50% CL:50% CH, and 75% CL:25% CH. The CL-CH mixtures were then mixed with ALGIN at either 0.5x (0.5% SA and 0.425% ECL) or 1x ALGIN (0.85% SA and 1% ECL). Each treatment was extruded into 69-mm-diameter casings and refrigerated at 3 °C for 48 h to allow gelation prior to being frozen to facilitate slicing into 5-mm-thick discs. A TA-HDplusC texture analyzer was used to evaluate (n=10 samples from each treatment per analysis). The shear force test was performed using a TA-42 probe to assess firmness and toughness, while 3-point-bend was conducted with a TA-43R probe to evaluate hardness, flexibility, and stiffness. Data were analyzed as a 2-way ANOVA using the GLIMMIX procedure of SAS. Least square means were separated at $P \leq 0.05$ with the PDIFF option. Firmness increased as CH and ALGIN concentrations increased ($P = 0.0349$). Increasing ALGIN increased treat toughness and treats containing 75% CH were the toughest compared with those containing 25 or 50% CH ($P < 0.0001$). Hardness increased as ALGIN inclusion increased, but there was no difference among the CL-CH combinations ($P = 0.0003$). Flexibility of treats with 75% CL was greater than those with 25 or 50% CL ($P = 0.0074$). Inclusion of 0.5x ALGIN produced more flexible treats than 1x ALGIN ($P = 0.0335$). Stiffness was unaltered by either CL-CH ratio or ALGIN concentration ($P = 0.0722$). Increasing ALGIN increased the firmness, toughness, and hardness of raw pet treats. Treats with a majority of CL were more flexible, while treats with 75% CH were toughest. Further work is necessary to determine if these alterations impact pet owner raw treat buying preferences.

Key Words: Alginate, shear force, meat processing, co-products, pet food

M24 Texture analysis of pet treats generated from pressure cooked broiler wing tips

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The broiler chicken industry produces numerous co-products that have the potential to be upcycled by the pet food industry. Wing tips (WT) are a co-product often sent to rendering after the wing is cut into the wingette, drumette, and WT. The objective was to evaluate the textural characteristics of pet treats generated from WT containing increasing ALGIN (sodium alginate (SA) + encapsulated calcium lactate (ECL)) inclusions as a structure forming component. Raw WT obtained from a commercial poultry plant were pressure cooked for 90 min at 55 kPa. Each pressure cooker (n = 4) contained 6,800 g of WT and 486 g water. After cooking, WT were drained, ground through a 4.76-mm grinder plate, and mixed with 1 of 4 different ALGIN inclusions: 0x (no ALGIN inclusion), 0.5x (0.425% ECL + 0.5% SA), 1x (0.85% ECL + 1% SA), and 2x (2% ECL + 1.7% SA).

Mixtures were stuffed into 63.5-mm diameter casings, stored overnight at 4 °C, and frozen (-20 °C) for 3 h to facilitate slicing into 5-mm-thick slices for analysis. After slicing, treats were held at 4 °C until all samples could be analyzed (< 48 h). Samples (n = 10 per treatment per analysis) were analyzed for 3-point-bend (stiffness, hardness, and flexibility) and shear force (SF; firmness and toughness) using a TA-HDplusC texture analyzer with a TA-43R and TA-42 probe, respectively. Data were analyzed as a 1-way ANOVA using the GLIMMIX procedure of SAS and least square means were separated using the PDIFF option at $P \leq 0.05$. Stiffness ($P = 0.2526$) and hardness ($P = 0.1719$) of treats were unaffected by ALGIN inclusion. Treats containing 0x ALGIN had the greatest flexibility values ($P < 0.0001$). Treats containing 0.5x and 2x ALGIN were less firm than treats without ALGIN ($P = 0.0057$). Toughness was greater in treats with 0x and 1x ALGIN inclusions compared with 2x ALGIN-containing treats ($P = 0.0402$). In conclusion, low-value, ground, pressure cooked WT from broiler processing were upcycled into pet treats. Textural characteristics of treats were differentially affected by increasing inclusion of a structure forming agent. However, it is unclear how these changes will impact consumer acceptability of treats.

Key Words: wing tip, sodium alginate, upcycling, texture analysis, pet treats

M25 Evaluation of textural characteristics of dehydrated pet treats generated using broiler wing tips and a structure forming agent

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Broiler processing co-products have the potential to be reallocated to value-added pet food markets where greater financial return may be possible. The pet treat industry is rapidly growing and could benefit from the upcycling of co-products such as wing tips (WT). Structure forming agents are often included in products to enhance textural characteristics. The objective of this experiment was to evaluate the toughness, firmness, stiffness, hardness, and flexibility of dehydrated jerky-style pet treats generated from WT containing increasing concentrations of a structure-forming agent, ALGIN (sodium alginate (SA) + encapsulated calcium lactate (ECL)). Raw, previously frozen WT were ground through a 4.8-mm grinder plate and mixed with 1 of 4 different ALGIN inclusions: 0x (no ALGIN inclusion), 0.5x (0.425% ECL + 0.5% SA), 1x (0.85% ECL + 1% SA), and 2x (2% ECL + 1.7% SA). Each mixture was extruded into 63.5-mm diameter casings, stored overnight at 4 °C to allow gelation, and frozen (-20 °C) for 3 h to facilitate slicing into 5-mm-thick slices for analysis. After slicing, dehydrated using a convection oven at 93 °C for 2.5 h, individually wrapped, and held at 4 °C until all samples could be analyzed. Samples (n = 10 per treatment per analysis) were analyzed for shear force (SF; firmness and toughness) and 3-point-bend (stiffness, hardness, and flexibility) using a TA-HDplusC texture analyzer with a TA-42 and TA-43R probe, respectively. Data were analyzed as a 1-way ANOVA using the GLIMMIX procedure of SAS and least square means were separated with the PDIFF option at $P \leq 0.05$. Treats containing 1x ALGIN were tougher ($P < 0.0001$) and firmer ($P < 0.0001$) than those from all other treatments. Stiffness was lower in treats with 1x and 2x ALGIN ($P < 0.0001$). Hardness was greater ($P < 0.0001$) and flexibility was lower ($P < 0.0001$) in treats produced with 0x and 0.5x ALGIN inclusion compared with 1x and 2x ALGIN-containing treats. These data indicate that textural characteristics of dehydrated jerky-style pet treats were impacted by varying inclusion of a structure forming agent containing SA and ECL. The optimal texture of a jerky-style pet treat has yet to be determined.

Key Words: sodium alginate, encapsulated calcium lactate, wing tip, processing co-product, upcycling

M26 Evaluation of textural characteristics of dehydrated pet treats generated from chicken liver and heart combinations Said Herrera¹IGS, Marc Pr  sum  ¹, Rigo Soler¹, Jorge Romero¹, Justin Dunavant¹, Moses Chilenje¹, Jorge Sandoval¹, Luis Avila¹, Catherine Odom¹, Laura Grner¹, Amit Morey¹, Robert Mason², Eric Altom², Charles Starkey¹ ¹Auburn University Department of Poultry Science, ²Balchem Corporation

Chicken livers (CL) and hearts (CH) are broiler processing co-products that often go unused for human consumption and can be undervalued. Low value co-products like these may be attractive to pet food manufacturers for use in pet treats. Purchasing decisions may be influenced by textural characteristics of pet treats. The objective was to evaluate textural characteristics of dehydrated pet treats generated from 3 different CL and CH mixtures with 2 different structure forming agent inclusions (ALGIN = sodium alginate (SA) and encapsulated calcium lactate (ECL)). Chicken livers and CH were ground through a 4.8-mm grinder plate and mixed at the following 3 ratios: 25% CL:75% CH, 50% CL:50% CH, and 75% CL:25% CH prior to being combined with 1 of 2 ALGIN concentrations: 0.5x ALGIN (0.5% SA + 0.425% ECL) or 1x ALGIN (1% SA + 0.85% ECL) to produce 6 treatments. Mixtures were extruded into 69-mm-diameter casings, refrigerated at 4   C for 48 h to allow gelation, and then frozen to facilitate slicing into 5-mm-thick discs. Sample discs were dehydrated in a Hobart HEC5D convection oven at 93   C for 2.5 h. Textural properties of dehydrated samples were evaluated using a TA-HDplusC texture analyzer. Samples (n = 10 per treatment per analysis) were evaluated for shear force (firmness and toughness) and 3-point bend (hardness, flexibility and stiffness). Data were analyzed as a 2-way ANOVA with the GLIMMIX procedure of SAS and least square means were separated using the PDIF option at $P \leq 0.05$. No interaction among CL:CH combination and ALGIN inclusion were observed for firmness ($P = 0.2258$). Treats containing 1x ALGIN exhibited decreased firmness compared with those including 0.5x ALGIN ($P = 0.0312$). Dehydrated treats containing 50%CL: 50%CH and 0.5x ALGIN were the hardest, the least flexible, and stiffest compared with all other treats ($P \leq 0.0004$). In conclusion, CL and CH were successfully repurposed into dehydrated pet treats using ALGIN as a structure-forming agent. Ratios of CL:CH and ALGIN inclusion interacted to differentially impact textural characteristics of dehydrated pet treats. It is unclear how this could impact treat acceptability and purchasing decisions by pet owners.

Key Words: Sodium Alginate, Calcium Lactate, Chicken Liver, Texture Analysis

M27 Analysis of instrumental color over time of raw pet treats generated from broiler chicken wing tips Jorge Romero¹IGS, Justin Dunavant¹, John Rogers¹, Christopher Almendares¹, Marc Presume¹, Said Herrera¹, Josh Renew¹, Jorge Sandoval¹, Jorge Banegas¹, Gerardo Abascal-Ponciano¹, Diego Ventura¹, Luis Guzman¹, Tristan Reyes², Madison Wagoner², Robert Mason³, Eric Altom³, Jason Sawyer², Jessica Starkey¹, Charles Starkey¹ ¹Auburn University Department of Poultry Science, ²Auburn University Department of Animal Science, ³Balchem Animal Nutrition and Health, Balchem Corp.

The pet treat market has expanded rapidly in recent years. Chicken wing tips (WT) are a broiler processing co-product with potential to be upcycled into value-added products like pet treats. Product color can influence purchasing decisions of pet owners. Therefore, the objective was to evaluate instrumental color over time of raw pet treats derived from WT containing 1 of 4 concentrations of a structure forming agent (ALGIN) containing sodium alginate (SA) and encapsulated calcium lactate (ECL). Previously frozen WT were ground through a 4.76-mm grinder plate. Ground WT were divided into 4 portions and mixed with 1 of 4 concentrations of ALGIN: 0x (no ALGIN), 0.5x (0.50% SL + 0.425% ECL); 1x (1.00% SL + 0.85% ECL), and 2x (2.00% SL + 1.70% ECL). Each mixture was stuffed into 63.5-mm-diameter casings, stored overnight at 4   C to facilitate gelation, and frozen at -20   C prior to slicing into 5-mm-thick slices and stored at 4   C for 7 d. Instrumental color was assessed by taking 3 surface

measurements with the CIE color space L^* , a^* , b^* color scale using a HunterLab MiniScan EZ 4500 spectrophotometer on each of 10 samples per treatment on d 0, 3, 5, and 7 of storage. Data were analyzed as a 2-way ANOVA using the GLIMMIX procedure of SAS. Treatment least square means were separated using the PDIF option at $P \leq 0.05$. Interactions between ALGIN concentrations and day were observed for lightness (L^*), redness (a^*) and yellowness (b^*) values. On d 0, 3, and 5 of storage, treats containing 2x ALGIN were lighter than those with 0x ALGIN, except on d 7 where they were similar ($P < 0.0001$). On d 0, 3, and 7, treats with 2x ALGIN were the reddest, whereas on d 5, 0.5x-ALGIN-containing treats had the highest a^* values ($P < 0.0001$). Treats with 2x ALGIN were yellowest on d 0, 3 and 5 compared with all other treatments; however, on d 7, treats containing 1x and 2x ALGIN were similar ($P < 0.0001$). Overall, increasing ALGIN concentration did not stabilize color in refrigerated raw pet treats stored for 7 d post-production.

Key Words: Pet treats, color, Sodium alginate, encapsulated calcium lactate, broiler chicken

M28 Effect of inclusion of a forming agent on chemical analysis of pet treats developed from broiler wing tips Jorge Sandoval¹IGS, Jonh Rogers¹, Jorge Romero¹, Justin Dunavant¹, Marc Presume¹, Said Herrera¹, Josh Renew¹, Jorge Banegas¹, Gerardo Abascal-Ponciano¹, Christopher Almendares¹, Diego Ventura¹, Luis Guzman¹, Tristan Reyes², Madison Wagoner², Robert Mason³, Eric Altom³, Jason Sawyer², Jessica Starkey¹, Charles Starkey¹ ¹Auburn University Department of Poultry Science, ²Auburn University Department of Animal Science, ³Balchem Animal Nutrition and Health, Balchem Corp.

Broiler chicken processing results in the generation of various low-value co-products such as wing tips (WT) that may be useful in the design of pet treats improve value. ALGIN is structure forming agent composed of sodium alginate (SA) and encapsulated calcium lactate (ECL). The objective was to assess the effect of different inclusions of ALGIN on chemical analysis of pet treats derived from pressure cooked (PC), raw, and raw then dehydrated chicken wing tip (WT). For PC treats, previously frozen WT were pressure cooked at 55 kPa for 90 min. Raw WT and cooked WT were ground separately using a 4.8-mm grinder plate. ALGIN was added and mixed with ground WT at 1 of 4 concentrations: 0.5x (0.50% SL + 0.425% ECL), 1x (1.00% SL + 0.85% ECL), and 2x (2.00% SL + 1.70% ECL) the manufacturer's recommended inclusion plus a control group with no ALGIN inclusion (0x). Products were then extruded into 63.5-mm diameter casings, stored at -20  C, and sliced into 5-mm thick discs. Raw WT slices were held at 93   C for 2.5 h to produce dehydrated pet treats (RD). Chemistry analyses were performed on PC, raw, and RD samples. Ten samples from each treatment were used for each of the following analyses: expressible moisture (EM; filter paper press method), pH, water activity (aW), and moisture content (MC). Data were analyzed as a 1-way ANOVA using the GLIMMIX procedure of SAS and means were separated at $P \leq 0.05$. EM on PC treats was similar for 0x and 1x treatments and increased for 0.5x and 2x ALGIN ($P < 0.0001$). EM on raw treats decreased as ALGIN inclusion increased for 0x, 0.5x, and 1x samples, and stabilized when 2x ALGIN was used ($P < 0.0001$). MC of raw treats was higher for 0x and 0.5x ALGIN and decreased in 1x and 2x ALGIN ($P < 0.0001$). Pressure cooked treats with 0x ALGIN had higher MC than 0.5 and 2x ALGIN while 1x treats had the lowest MC ($P < 0.0001$). Water activity and pH were similar among all ALGIN inclusions in raw and PC WT treats ($P > 0.05$). Raw dehydrated samples with 1x ALGIN had greater aW than 0.5x and 0x ALGIN ($P = 0.0018$). In RD samples, MC increased as ALGIN inclusion increased ($P < 0.0001$). Both raw and pre-cooked ground WT were successfully used to produce pet treats. As expected, ALGIN inclusion altered the EM and MC of WT-derived treats.

Key Words: alginate, chicken wing tips, broiler processing co-products, chemistry

M29 Analysis of instrumental color over time in pet treats derived from pressure-cooked broiler chicken wing tips Christopher Almendares^{*1GS}, Justin Dunavant¹, Jorge Romero¹, John Rogers¹, Marc Presume¹, Said Herrera¹, Josh Renew¹, Jorge Banegas¹, Gerardo Abascal-Ponciano¹, Diego Ventura¹, Jorge Sandoval¹, Luis Guzman¹, Tristan Reyes², Madison Wagoner², Robert Mason³, Eric Altom³, Jason Sawyer³, Jessica Starkey¹, Charles Starkey¹ ¹*Auburn University Department of Poultry Science*, ²*Auburn University Department of Animal Science*, ³*Balchem Animal Nutrition and Health, Balchem Corp.*

Chicken wing tips (WT) are a low-value broiler processing co-product with potential for conversion into value-added pet treats. Pet owner purchasing decisions can be influenced by product color. The objective was to evaluate instrumental color over time of pet treats generated from pressure-cooked WT containing different inclusions of a structure forming agent (ALGIN) composed of sodium alginate (SA) and encapsulated calcium lactate (ECL). Previously frozen, chicken WT were pressure cooked at 55 kPa for 90 min, ground through a 4.8-mm grinder plate, and mixed with 1 of 4 ALGIN concentrations: a control (0x) without ALGIN, 0.5x (0.5% SA and 0.425 ECL), 1x (1% SA and 0.85% ECL), and 2x (2% SA and 1.70% ECL). Product was extruded into 63.5-mm-diameter casings, stored overnight at 4 °C, and frozen at -20 °C prior to slicing into 5-mm-thick discs for analysis. A Hunter Lab Miniscan XE Plus colorimeter was used to measure surface color (CIE L* (lightness), a* (redness), b* (yellowness)) in triplicate on each disc after 0, 3, 5, and 7 d of storage post-slicing at 4 °C. Data were analyzed as a 2-way ANOVA with the GLIMMIX procedure of SAS and MS were separated at $P \leq 0.05$ with the PDIF option. No interaction among ALGIN and time (in d) for sample lightness was observed (L^* ; $P = 0.1036$). Lightness increased from d 0 to 7 ($P < 0.0001$). With increasing ALGIN concentrations, a quadratic effect was observed in lightness ($P < 0.0001$). Interactions were observed among ALGIN concentration and time for redness and yellowness ($P \leq 0.0088$). Redness decreased over time ($P < 0.0001$). On d 0, 3, and 5, treats containing 2x ALGIN had the highest a* values ($P < 0.0001$). On d 7, in treats containing 0x, 0.5x, and 1x ALGIN, a* values decreased linearly ($P < 0.0001$). At all time points, a quadratic effect of ALGIN concentration was observed ($P < 0.0001$). Treat yellowness (b*) increased as ALGIN increased from 0x to 1x but treats containing 2x ALGIN were similar to those with 0.5x ALGIN ($P = 0.0088$). In conclusion, low value, pre-cooked, ground WT were successfully combined with a structure forming agent to produce a pet treat. Increasing ALGIN inclusion impacted treat color over 7 d of storage time, though it is unclear how this may influence pet owner buying preferences.

Key Words: alginate, broiler chicken processing, instrumental color, pet food

M30 Effect of forming agent inclusion on textural profile analysis of pet treats generated from broiler wing tips Diego Ventura Urbina^{*1UG}, Justin Dunavant¹, Jorge Romero¹, John Rogers¹, Marc Presume¹, Jorge Sandoval¹, Said Herrera¹, Josh Renew¹, Jorge Banegas¹, Gerardo Ponciano Abascal¹, Christopher Almendares¹, Luis Guzman¹, Tristan Reyes², Madison Wagoner², Robert Mason³, Eric Altom³, Jason Sawyer², Jessica Starkey¹, Charles Starkey¹ ¹*Auburn University Department of Poultry Science*, ²*Auburn University Department of Animal Science*, ³*Balchem Animal Nutrition and Health, Balchem Corp.*

Processing chicken generates different co-products with reduced commercial value. Wing tips (WT) are the most distal portion of the wing and are obtained by cutting through the carpal joint. Forming agents are often used in ground meat-containing products to provide structure. The objective was to assess the effect of different inclusions of forming agent (ALGIN; sodium alginate (SA) and encapsulated calcium lactate (ECL)) on texture profile analysis (TPA) of pet treats generated from cooked WT. Previously frozen WT were pressure cooked at 55 kPa for 90 min, drained, and ground through a 4.8-mm grinder plate. Ground WT were mixed with 4 different ALGIN inclusions: 0x (no ALGIN inclusion), 0.5x (0.425% ECL + 0.5% SA), 1x (0.85% ECL + 1% SA), and 2x (2% ECL + 1.7% SA). After mixing each treatment was extruded into 63.5-mm diameter casings stored overnight at 4 °C, and frozen (-20 °C) for 3 h to facilitate slicing into 5-mm-thick discs for analysis. After slicing, treats were held at 4 °C until all samples could be analyzed (< 48 h). A TA-HDplusC texture analyzer with a two-cycle compression and a TA-43R cylinder probe was used to measure 6 attributes: hardness, maximum force required to deform; cohesiveness represents structural integrity under compression; adhesiveness is adhesion of sample to the probe; chewiness is energy required to masticate; resilience is ability of sample to regain original form; springiness is height that the sample recovers between mastication. Data were analyzed as a 1-way ANOVA using the GLIMMIX procedure of SAS and least square means were separated at $P \leq 0.05$. No differences in treat cohesiveness, chewiness, resilience, or springiness were observed among treatments ($P \geq 0.0886$). Treats containing 0x ALGIN were hardest among all treatments and required the highest force to deform the treat ($P \leq 0.01$). Adhesiveness was also greatest in treats without ALGIN ($P \leq 0.04$). In conclusion, inclusion of more than 0.5x ALGIN did not result in changes in TPA of pet treats produced from cooked, ground broiler WT. It was also demonstrated that cooked WT can be used successfully as a primary ingredient in combination with a forming agent (ALGIN) to produce pet treats.

Key Words: wing tip, sodium alginate, texture analysis, pet treats, TPA

Pathology

M31 Pathogenesis of *Histomonas meleagridis* in broilers Vijay Durairaj^{*1}, Ryan Vander Veeb¹, Mary Drozd² ¹*Huvepharma, Inc.*, ²*University of Nebraska-Lincoln*

Histomonas meleagridis causes histomoniasis (blackhead disease) in gallinaceous birds. In recent years, increased incidences of histomoniasis have been reported in broilers and broiler breeders. The main objective of this study was to understand the pathogenesis of *H. meleagridis* in chickens. A wild-type *H. meleagridis* isolate was evaluated in broiler chickens. Twenty-six commercial broilers were enrolled in a study and divided into two groups. At 17 days-of-age, one group was challenged with 3×10^5 *H. meleagridis* by intra-cloacal inoculation and the other group served as negative control. Necropsy was performed at 35 days-of-age and 100% incidence of histomoniasis was noticed in chickens challenged with *H. meleagridis* isolate. In addition to the characteristic gross lesions in ceca and liver, other lesions such as splenomegaly, hepatomegaly, pericarditis and airsacculitis were also reported. Histopathology confirmed the presence of

histomonads in affected tissues. Wild-type *H. meleagridis* isolate resulted in clinical signs, systemic infection and mortalities in broiler chickens.

Key Words: Histomoniasis, Blackhead disease, *Histomonas meleagridis*, Pathogenesis, Chickens

M32 Understanding the transmission of *Histomonas meleagridis* in turkeys Catherine Fudge^{*GS}, Olivia Wedegaertner, Katherine Cupo, Christina Sigmon, Frank Edens, Robert Beckstead, Chongxiao Chen *North Carolina State University*

Histomoniasis outbreaks are initiated by introduction of *H. meleagridis* to turkey flocks and propagated via lateral transmission. It is essential that *H. meleagridis* transmission routes and factors are understood to control mortality. Past experiments have not produced significant lateral transmission of *H. meleagridis*; however, a single case of lateral transmission was observed in birds fed a low protein high-fat diet (16% protein, 10% fat).

In study I, dietary treatments of T1 (low protein, high fat), T2 (T1 stored in high heat for 2 weeks), and T3 (low protein, high fat with aflatoxin-contaminated ingredients) were assigned to 180 5-week-old turkey hens (3 reps x 20 birds) raised in floor pens. The hens were cloacally infected with 1mL of 100,000 histomonads/bird at 2 days post-placement. A secondary infection was performed at 11 days post-infection (DPI). At 32 DPI, histomoniasis lesion scores were recorded for all birds. The data were analyzed using PROC GLM in SAS 9.4 ($P < 0.05$). No lateral transmission was observed during this trial and differences in mortality and morbidity between treatments were statistically insignificant ($P = 0.1012$ and $P = 0.2312$, respectively). Study II assessed the ability to infect turkeys repeatedly orally with *H. meleagridis* to induce histomoniasis, a method not previously studied. Ninety male poults were allocated to 3 treatments (3 reps x 10 birds): T1 (cloacal infection with *H. meleagridis* in culture media), T2 (oral infection with *H. meleagridis* in culture media), and T3 (oral infection with *H. meleagridis* in cecal content). Poults were infected twice daily with 50,000 histomonads/bird at 8 am and 5 pm for 5 days starting at 14 days old. Cloacal infection caused 100% morbidity and oral infection with *H. meleagridis* cecal content mixtures produced 43% morbidity compared to 8% morbidity when orally infected with pure culture ($P = 0.006$). In summary, high fat, low protein diets do not lead to significant transmission of *H. meleagridis*. Furthermore, oral infection with *H. meleagridis* may result in higher morbidity, suggesting that oral transmission of *H. meleagridis* is plausible. Further research is needed to understand if *H. meleagridis* is naturally transmitted orally.

Key Words: Blackhead, Turkey, Transmission, Diet, Histomonas

M33 A research model to investigate the role of stressors on *Cochlosoma anatis* infections in turkeys Christina Sigmon*, Robert Beckstead, Chongxiao Chen *NC State University*

Cochlosoma anatis is a flagellated protozoa that has been associated with enteritis, poor body weight, and mortality in turkeys. Currently, there are no treatments available. The role of stressors in infections and interactions between *C. anatis* with other enteric parasites are unclear. A *C. anatis* research model was established for testing feed additives to reduce the parasite load and understand the effects of stressors and presence of other parasites on disease signs. 300 day-old Nicholas male turkey poults were placed in 30 isolator cages (3 reps x 10 birds). The experiment adopted 2 x 5 factorial design; with or without *C. anatis* infection and 5 stressor treatments: 1) Control; 2) high energy diet; 3) *Tetratrichomonas gallinarum* co-infection; 4) *Coccidia* co-infection; 5) Cold stress, D7-D14. Birds were challenged on D7 with 500,000 *C. anatis*/bird and the trial was terminated on D21. The birds were weighed at D7, D14 and D21. *C. anatis* were harvested and counted at D21. This was repeated with 8 birds/pen for trial 2. All data were analyzed statistically by using SAS software ($P < 0.05$). All performance data were analyzed by 2-way ANOVA. The *C. anatis* counting data were analyzed by one-way ANOVA. Interactions were found in the study. The high energy diet had lower mortality and higher body weight compared to the rest of treatments in both trials. Inconsistencies in mortality and body weights were seen in the 2 trials. In trial 1, the *C. anatis* control had 60% mortality vs. 37.5% in trial 2. Also, *T. gallinarum* and *Coccidia* co-infection group had lower mortality compared to infected control group in trial 1, but no differences were shown in trial 2. The *C. anatis* counts were higher in the high energy diet, cold stress, and *Coccidia* co-infection compared to control for both trials. In summary, high energy diets could reduce the mortality and maintain body weight in infected flocks. Further research is needed to investigate interactions between *C. anatis* and other protozoa and the environmental stressors that lead to clinical signs of disease. The stressors tested in the two trials suggested that the severity of *C. anatis* outbreaks observed during production may be related to the different stressors that turkeys have at various farms.

Key Words: *Cochlosoma*, turkey, protozoa, stress

M34 Effects of *E. tenella* infection on growth performance, gut health, and volatile fatty acid production in broiler chickens Janghan Choi^{*1GS}, Hanseo Ko¹, Yuguo Thompkins¹, Po-yun Teng¹, Jeferson Lourenco², Todd Callaway², Woogyun Kim¹ ¹*Department of Poultry Science, University of Georgia*, ²*Department of Animal and Dairy Science*

Eimeria tenella (*E. tenella*) is one of the poultry *Eimeria* spp. that damage cecal tissue in chickens. The purpose of the study was to investigate effects of different inoculation dosages of *E. tenella* on growth performance, gastrointestinal permeability, oocyst shedding, intestinal morphology, fecal consistency, ileal apparent digestibility, antioxidant capacity, and cecal volatile fatty acid production in broiler chickens. Five different dosages (T0: 0, T1: 6,250, T2: 12,500, T3: 25,000, and T4: 50,000) of *E. tenella* oocysts were inoculated via oral gavage to fourteen-day-old broilers. All groups were compared using the PROC MIXED in a completely randomized design followed by Tukey's comparison test. Orthogonal polynomial contrasts were utilized to evaluate the significance of linear or quadratic effects of different *E. tenella* inoculation dosages. Inoculation of *E. tenella* linearly increased FCR ($P < 0.05$), and feed intake tended to be quadratically increased on 6 days post-infection (dpi; $P = 0.08$) and 7 dpi ($P = 0.09$). Cecal lesion score of each treatment was T0: 0; T1: 0.39 ± 0.14 ; T2: 0.93 ± 0.21 ; T3: 1.25 ± 0.16 ; and T4: 1.58 ± 0.2 . Cecal total VFA production was linearly reduced due to *E. tenella* infection on 6 dpi ($P < 0.01$). *E. tenella* infection deepened cecal crypts depth (CD) on 6 dpi ($P < 0.05$). Gastrointestinal permeability tended to be linearly increased ($P = 0.07$). *E. tenella* infection tended to linearly reduce duodenal VH ($P = 0.1$) and jejunal VH on 9 dpi ($P = 0.09$). Different dosages of *E. tenella* modulated trends of fecal moisture content and oocyst shedding. Therefore, *E. tenella* infection impaired feed efficiency and small intestinal health mainly by reducing cecal VFA production and absorption in broilers.

Key Words: *Eimeria tenella*, broiler chicken, gut health, volatile fatty acids, feed efficiency

M35 Visualizing *Eimeria acervulina* infection in duodenal tissue using in situ hybridization Sara Cloft^{*1GS}, Kate Miska², Eric Wong¹ ¹*Department of Animal and Poultry Sciences Virginia Tech*, ²*Animal Biosciences and Biotechnology Laboratory, Henry A. Wallace Beltsville Agricultural Research Center*

Infection with the protozoan parasite *Eimeria acervulina* causes lesions in the duodenum as the parasite invades epithelial cells lining the villi. Traditionally, invading oocysts can be identified visually in hematoxylin and eosin-stained tissue based on their unique morphological characteristics. These techniques, however, rely on trained personnel and can not be seen without high magnifications. In situ hybridization (ISH) methodology allows for the visualization of unique mRNA sequences within fixed tissue. Previous research has utilized ISH to differentiate cell types along the chicken intestine and for detection of viral pathogens in mice and human tissues. This objective of this study was to evaluate the presence or absence of *E. acervulina* sporozoite surface antigen gene through quantitative PCR and then to utilize ISH to visualize *E. acervulina* through a sporozoite surface antigen gene, within the duodenal tissue of broiler chickens following infection with 100,000 *E. acervulina* oocysts at 21 d of age. The *E. acervulina* sporozoite surface antigen gene was only detectable on 5 and 7 d post-infection in both ISH and quantitative PCR for 6 out of 6 birds infected with *E. acervulina* and not detected in any of the uninfected birds for any timepoint (6 per treatment, timepoint). Further, ISH of *E. acervulina* can be compared with ISH of cell marker genes or histochemical stains to better understand how *E. acervulina* invades intestinal tissue. Serial sections stained with *E. acervulina* or Mucin 2 by ISH and counter-stained with Alcian blue and hematoxylin showed that *E. acervulina* was not present in blue-stained goblet cells containing acidic mucins. It has been previously suggested that acidic mucin has stronger defensive barrier functions than neutral mucins; therefore, it may prevent sporozoite penetration. This is the first study to utilize ISH for the detection of *Eimeria* spp. in chickens. This methodology has the potential to

investigate localized gene expression changes and generally improve our understanding of the pathophysiology behind *Eimeria* infection.

Key Words: *Eimeria* acervulina, sporozoite surface antigen, in situ hybridization, coccidiosis, goblet cells

M36 Saponin-based solution (Norponin XO) as efficient as coccidiostats to maintain broilers' zootechnical performances under coccidiosis challenge. Mohammed El Amine BENARBA^{1,2}, Pierre CHICOTEAU^{1,2} ¹NOR FEED, ²LABCOM FEED IN TECH: Université d'Angers

Gut health is a pillar of animal welfare and productivity. In broilers, gut health can be negatively affected by coccidiosis. To limit the effect of this disease, coccidiostats have been used successfully for decades. However, resistance is often a side effect of their extensive use. In addition, consumer demand for antibiotic-free poultry products is increasing. This demand considerably limits the range of molecules available to control coccidiosis for this type of production. Thus, a natural and efficient tool to help modern poultry producers meet productivity needs and market demand is more than necessary. Saponin-rich plants such as *Yucca schidigera* and *Trigonella foenum-graecum* are promising tools. The objective of this presentation is to share results from different evaluation methods applied to assess the efficiency of Norponin XO 2 (NPXO2), saponin-rich plant-based solution, and to provide a clear picture of the alternatives available for gut health management. From 2016 to 2021, 2 types of experimental designs were applied. The first consisted of experimental infestations within research facilities. In this experimental design, 4 groups of birds were used: infested-untreated control (IUC), untreated-uninfested control (UUC), infested-coccidiostats treated (positive control group), and infested-NPXO2 treated (NPXO2). The second experimental design was conducted on commercial farms with 2 groups, one with "conventional" coccidiosis management tools and the other supplemented with NPXO2. In both designs, production performance parameters were monitored and gut health was assessed using the Johnson and Reid method of scoring coccidiosis-related intestinal lesions (ILS). The results of the first set of experiments showed that coccidiostats and NPXO2 supplements were able to reduce ILS related to *Eimeria* spp. infestation and maintain zootechnical performance equally. In the second set of experiments, in addition to the fact that NPXO2 supplementation was as efficient as conventional tools in managing coccidiosis under field conditions, NPXO2 birds exhibited improved livability. These results suggest that NPXO2 supplementation is a reliable tool for coccidiosis management. Therefore, providing agility to support efficient and sustainable poultry production.

Key Words: Coccidiosis, saponines, feed additives, botanicals

M37 Two blends of botanical extracts optimize coccidiosis-preventive strategies and maintain growth performance in Eimeria-challenged broilers. Jonathan Pierron^{1,2}, Bertrand Medina³, Ivan Girard³, Carl Julien^{1,2} ¹Université Laval, ²Centre de recherche en sciences animales de Deschambault (CRSAD), ³Probiotech International Inc.

Two botanical extract blends differentiated from their phenylpropanoid, phenolic and saponin contents were evaluated in *Eimeria*-challenged broilers (55 birds/pen, 9 replicates). Trial#1 involved 2,475 Ross 308 males assigned to: 1) non-infected (NI); 2) infected (IF); 3) IF+decoquinat/zonalene shuttle program (DZ); 4) IF+250 ppm Synbiotec® CVA (SYN250); 5) IF+500 ppm (SYN500). In trial#2, 1,980 *Eimeria*-vaccinated (IM-MUCox3®, CEVA) birds were assigned to: 1) NI; 2) IF; 3) SYN250; 4) IF+250 ppm Alterna® OP (AOP). For both trials, *Eimeria* infection was induced by an oral administration of 2×10^5 sporulated oocysts at d14. Data were analyzed by a mixed model including treatment and barn section as fixed and random effects, respectively, and by Kruskal-Wallis tests. In trial#1, DZ palliated the *Eimeria* infection impacts on growth performance at d21 and d34, mortality rate overall, oocysts per gram of feces (OPG) at d20 and *E. acervulina* and *E. tenella* intestinal lesions at d21. SYN500

increased BW at d34 (2.34 vs 2.26 kg, $P=0.0195$) and SYN250 showed a trend (2.31 vs 2.26 kg, $P=0.0904$), both decreased mortality rate overall (5 vs 14%, $P<0.05$), and increased total liveweight produced per pen at d34 in a dose-dependent manner (SYN250: 94.0 vs 82.9 kg, $P=0.0076$; SYN500: 95.3 vs 82.9 kg, $P=0.0033$), without affecting FCR, compared to IF. In trial#2, the *Eimeria* challenge (IF) also reduced BW at d20 (734 vs 780 g, $P=0.0456$), worsened FCR during 10-20d (1.50 vs 1.37, $P=0.0478$), levels of OPG at d20 (6.2 vs 5.6 log₁₀, $P<0.0001$) and intestinal *E. tenella* score at d21 (1.33 vs 0.22, $P=0.0002$), compared to NI. SYN250 increased BW at d10 (251 vs 242 g, $P=0.0012$) and at d20 (790 vs 734, $P=0.0443$), and improved FCR during 0-10d (1.17 vs 1.23, $P=0.0001$) and 10-20d (1.36 vs 1.50, $P=0.0261$), compared to IF. AOP showed a trend to improve FCR during 0-20d (1.32 vs 1.39, $P=0.0777$) and overall (1.31 vs 1.33, $P=0.0599$). SYN250 and AOP did not affect OPG or intestinal lesions, compared to IF. Overall, SYN, designed for standard production, and AOP, compatible with National organic standard (NOS), both enhance growth performances in *Eimeria* vaccination conditions and could be introduced to any coccidiosis control strategies without anticoccidial drugs.

Key Words: Conventional production, Organic production, Coccidiosis, Botanical extracts, Broilers

M38 Comparison of two Clostridium perfringens strains for inducing subclinical necrotic enteritis in broiler chickens Nima Emami¹, Rami Dalloul *Department of Poultry Science, University of Georgia*

Subclinical necrotic enteritis (NE) is responsible for the greatest economic impact on poultry production. However, several research models lead to clinical NE with high mortality, which often does not reflect field conditions. Thus, we compared *Clostridium perfringens* (Cp) strains #4 and #6 in an infection model with *Eimeria maxima* (EM) to induce a subclinical NE. A total of 33, day (d)-old broilers were individually wing-tagged and raised till d 14 when they were allocated to one of four treatment groups: 1) non-challenged control (n=4); 2) EM (n=9); 3) EM+Cp#4 (Cp4; n=10); 4) EM+Cp#6 (Cp6; n=10). Birds were orally gavaged with 1 mL of 3,000 oocysts of EM on d 14. Birds in Cp4 and Cp6 groups were orally gavaged with 1 mL of Cp cultures on each of d 19 and d 20 ($\sim 1 \times 10^8$ CFU/mL). Birds were individually weighed on d 14, 19, 20, and 21. On d 21, blood was collected to measure mRNA abundance of C-C motif chemokine ligand (CCL)-4, CCL5 and CCL20, CARD domain containing (NLRC)-3 and NLRC5 inflammasome, and leucine-rich repeat and pyrin domain containing (NLRP)-3 inflammasome. Also, birds were necropsied to score coccidiosis and NE lesions. Data were analyzed using JMP (Pro16) and significance ($P<0.05$) between treatments were determined by LSD test. The only mortality, which was due to NE, occurred in Cp6 (2 birds) on d 21. BWG (d 14-21) was numerically lower in EM (371 g), Cp4 (378 g), and Cp6 (351 g) compared to NC (420 g). Cp4 and Cp6 had significantly higher NE lesion scores in the duodenum; but only Cp6 had greater scores in the jejunum compared to NC. Also, Cp6 had higher coccidiosis lesion scores in the duodenum compared to EM and NC; while in the jejunum and ileum coccidiosis lesion scores were higher in all challenged groups compared to NC. Cp6 had greater mRNA abundance of CCL20 compared to NC, CCL5 compared to Cp4 and EM, and NLRP3 compared to all groups. Overall, Cp#6 led to mortality and affected mRNA abundance of inflammasomes and chemokines in the circulation, while Cp#4 did not affect these parameters. Furthermore, the negative impacts of Cp#4 on BWG, and lesion severity were lower than Cp#6. Therefore, our infection model with EM and Cp#4 is a promising method for inducing subclinical NE, while Cp#6 could be used for the induction of clinical NE.

Key Words: Necrotic enteritis, *Clostridium perfringens*, *Eimeria maxima*, chemokine, inflammasome

M39 Essential oil and yeast cell wall efficacy in a necrotic enteritis challenge study Charles Hofacre¹, Liliana Borges², Melina Bonato², Ricardo Barbalho² ¹*Southern Poultry Research Group, Inc.*, ²*ICC Industrial Comércio Exportação e Importação SA*

The objective of this study was to determine the effectiveness of an essential oil associated or not with a yeast cell wall [YCW] to reduce the negative effects of necrotic enteritis [NE] on broilers. For this study, 420 one-day-old Ross x Ross male chicks were distributed in a CRB design with 6 treatments and 7 blocks using 10 birds per cage (0.44 m²/bird). The treatments were: Negative Control [NC] (no additive supplemented), Positive Control [PC] (Maxiban® 72 g/US ton), Essential Oil [EO300] (based on carvacrol 300 g/MT), Essential Oil [EO600] (600 g/MT), EO300 + YCW from *Saccharomyces cerevisiae* (IMW50® 0.5 kg/MT), EO600 + YCW (0.5 kg/MT). The challenge model consisted of coccidia from the *E. maxima* on day 14 and *Clostridium perfringens* [CP] gavaged on days 19 and 20 (with 1.0 mL of a 1.0 x 10⁸ CFU/mL). Because desired mortality was reached birds were not gavaged on day 21. On day 22 one bird per pen was humanely euthanized, weighed, necropsied, and lesion scored according to the following scale: 0=Normal, 1=Slight mucus covering small intestine, 2=Necrotic small intestine mucosa, 3=Sloughed and blood small intestine mucosa and contents. All birds were weighed at 0, 14, 22, and 28 days. Feed intake was monitored. The statistical analysis was performed (STATISTIX) using ANOVA with a comparison of means using LSD at 0.05. The NE lesions were compared using Kruskal-Wallis one-way non-parametric analysis of variance at 0.05. The CP challenge was greater than expected with 37%(a) NE mortality in the NC groups. However, all the treatments significantly reduced NE mortality. The PC had the greatest reduction (20b); followed by EO300+YCW (27.14ab), EO300 (28.57ab), EO600+YCW (28.57ab), and EO600 (32.86ab). As often occurs when NE mortality is high, there was not a significant difference in NE lesion scores, as was found in this trial. At 28 d the EO300+YCW had higher significant BWG (1.38a kg) compared to NC (1.29b) and PC (1.31b). The EO300+YCW had an FCR very similar (1.48b) to the PC (1.49ab) and significantly better than EO600+YCW (1.56a). Overall, with very strong CP, all the non-antibiotic treatments were effective in lowering NE mortality. These treatments also supported the birds' intestines and allowed them to convert feed to body weight efficiently.

Key Words: Coccidia, *Clostridium perfringens*, Poultry, Gut lesions, Mortality

M40 Effects of *Quillaja saponaria* extract on mRNA abundance of tight junction proteins and cellular metabolism genes during a necrotic enteritis challenge in broilers Candice Blue¹GS, Nima Emami¹, Mallory White², Omar Gutierrez³, Staci Cantley³, Rami Dalloul¹ ¹*University of Georgia*, ²*Virginia Western Community College*, ³*Huvepharma Inc.*

Necrotic enteritis (NE) is an intestinal disease that results in poor performance, inefficient nutrient absorption, mortality, and a devastating economic impact on poultry production. This study evaluated the effects of *Quillaja saponaria* extract on performance, intestinal lesion scores, mRNA abundance of tight junction proteins (TJP) including occludin (OCLN), zonula occluden 1 (ZO1), junctional adhesion molecule 2 (JAM2), and key mediators of cellular metabolism including peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC1-α). A total of 1,500 day (d)-old male broilers were allocated to one of five treatment groups (10 replicate floor pens, 30 birds/pen). Negative control (NC) group fed a corn-soybean meal diet; positive control (PC) fed NC + 50 g/MT BMD; and 3 additives (Clarity-Q) groups CQ15, CQ30, CQ60 given NC + *Quillaja saponaria* added at an inclusion rate of 15, 30, and 60 g/MT, respectively. A naturally occurring NE model was used by spraying a concentrated coccidiosis vaccine onto the feed and litter 24 h post-placement. On d 8, three birds/pen were necropsied for NE lesions and on d 8 and 14, jejunal samples were collected to assess mRNA abundance. On d 8, 14, and 42, body weight (BW) and feed intake (FI) were recorded, and feed conversion ratio (FCR) was calculated. The data were analyzed using

ANOVA (JMP, Pro 16) and significance ($P \leq 0.05$) was determined by the LSD test. Compared to PC and NC, S30 had numerically higher BW; while FI, FCR and mortality were numerically lower. Lesion severity was numerically lower in S30 compared to PC and NC. mRNA abundance of OCLN was significantly greater in S30 ($P < 0.05$) compared to both PC and NC. ZO1 mRNA abundance was significantly lower in PC when compared to all treatments, while JAM2 abundance was comparable to S30. PGC1-α mRNA abundance in S30 was also significantly greater than in NC birds. Overall, there was a positive association of JAM2 and ZO1 mRNA abundance with lesion severity, while OCLN and PGC1-α had a negative association. Adding *Quillaja saponaria* extract to the diet at 30g/MT has the potential to modify the gut barrier via TJPs, which could help birds better fight off infection.

Key Words: Necrotic enteritis, broiler, performance, tight junction, *Quillaja saponaria*

M41 Local and Systemic Cyto(Chem)kine and FGF expression Profile in Bacterial Chondronecrosis with Osteomyelitis (BCO)-affected Broilers Alison Ramser^{1,2}GS, Elizabeth Greene¹, Robert Wideman¹, Sami Dridi^{1,2} ¹*University of Arkansas, Center of Excellence for Poultry Science*, ²*University of Arkansas, Cell and Molecular Biology*

An organism's response to a disease or infectious state, such as bacterial chondronecrosis with osteomyelitis (BCO), involves both physiological symptoms like lameness as well as complex systemic immune responses. For the modern broiler (meat-type) chickens, BCO is a leading cause of lameness and rising threat to animal welfare and production. It involves bacterial infection, inflammation, and bone attrition with a poorly defined etiology. It is critical to define the key inflammatory and bone-related factors involved in BCO etiology. In this study, the local bone and systemic blood profile of inflammatory modulators, cytokines, and chemokines, was elucidated along with inflammasomes and key FGF genes. Increased expression of IL-1β was seen in BCO-affected bone, while BCO-affected blood showed upregulated TNFα and IL-12. The chemokine profile revealed increased IL-8 expression in both BCO-affected bone and blood in addition to inflammasome NLRC5 being upregulated in circulation. The key FGF receptor FGFR1 was significantly downregulated in BCO-affected bone. Exposure of human fetal osteoblast cells (hFOB) and chicken primary chondrocytes to plasma from BCO-affected birds as well as recombinant TNFα resulted in significantly decreased cell viability. These results demonstrate a unique signature of pro-inflammatory and bone-resorptive factors in BCO and their potential contribution to its etiology by impacting bone cell viability.

Key Words: lameness, cytokines, BCO, broilers, FHN

M42 Causes of morbidity and mortality in Alabama commercial broiler chickens Hailey Quercia¹GS, Carey Laster², Erfan Chowdhury² ¹*Auburn University College of Veterinary Medicine, Department of Pathobiology*, ²*Alabama Department of Agriculture and Industries, Veterinary Diagnostic Laboratory system*

The purpose of this project was to identify common causes of morbidity and mortality within the Alabama broiler population, and to explore the influence of season and complex location on the diagnosis of health issues.

A retrospective case series was performed using necropsy records from the Alabama State Diagnostic Laboratory System. To be included in the study, submissions had to be made in the calendar year 2019, be identified as broiler chickens and billed as a commercial submission, to have originated from complexes within Alabama, and be greater than 7 days of age. Descriptive statistics were performed using Microsoft Office Excel 365. Analytical statistics were performed using IBM SPSS statistics 27.

One-hundred and ninety-one broiler submissions were analyzed, representing more than 1,700 birds. Most submissions were made in the summer, and the least in the fall. The average age was 22.7 days, with the

most common age being seven days. From north Alabama, the top five identified causes of morbidity and mortality were aerobic bacterial infection/septicemia, femoral head necrosis, bursal atrophy, moderate to severe *Eimeria maxima* infection, and joint synovitis, respectfully. From south Alabama, the top five causes of morbidity and mortality were aerobic bacterial infection/septicemia, necrotic enteritis, upper respiratory disease, viral infection, and inclusion body hepatitis, respectfully. Other differences were identified, with north Alabama submissions having significantly higher average *Eimeria maxima* scores than south Alabama ($P=.027$). Differences were also seen in diagnoses made in different seasons, with necrotic enteritis more commonly diagnosed in the fall and winter, and joint synovitis more commonly diagnosed in the spring and summer.

This study identifies primary diseases and health conditions in submitted Alabama broilers and could be used to inform both diagnostic strategies and management. In the differences seen in diagnoses related to location and season, the regional differences appeared most dramatic, with north and south Alabama only having one condition in common in their top five causes of morbidity and mortality. Altogether, these findings underscore the importance of understanding health challenges on a local level.

Key Words: Broilers, pathology, Alabama, mortality, septicemia

M43 Survey of feed mills around United States for select bacterial pathogens Cesar Escobar Lobo^{*GS}, Ken Macklin, Wilmer Pacheco, Rudiger Hauck *Auburn University*

Feed is one of the principal sources of microbial contamination in animal production industry. The following project has as an objective to analyze and determine the microbial content of feed ingredients and finished feed from different feed mills around the United States focusing on *Salmonella*, *E. coli*, *Clostridium perfringens* and *Clostridium argentinense*. Additionally, tris phosphate carbonate (TPC) and buffer peptone water (BPW) were evaluated as pre-enrichment mediums for detection of *Salmonella* in feed, with an initial pH of 8 and 7 respectively. Samples were collected from 4 of 8 commercial feed mills (letter A, B, C and D assigned), a total of 136 samples (38 ground corn, 20 corn DDGS, 30 post mixing, 28 finished feed loadout, 10 wheat midds and 10 post cooling). Using selective media, the samples were assayed for *Salmonella*, *E. coli*, *C. perfringens*, anaerobic and aerobic colony counts. For the pre-enrichment evaluation, pH was measured at 0 and 24 hrs, later transferred to Tetrathionate Brilliant Green Broth (TTB) as enrichment for 24 hrs and subsequently plated on selective media to confirm positive or negative presence of *Salmonella*. The results (colony forming units) were \log_{10} transformed and analyzed using ANOVA (significant $P<0.05$), means were separated using Tukey HSD in SPSS® software. No *Salmonella* colonies were recovered from TPC and BPW and no statistical differences were observed with *E. coli* and aerobic colony counts (PCA) between samples. *Clostridial* spp. colony counts were below the reportable limit in most of the samples. However, fourteen suspect *C. perfringens* colonies were isolated and stored for further analysis. Feed mills A, C, and D reported no significant differences in bacterial counts. However, differences ($P<0.05$) were observed with TPC and BPW as pre-enrichment, post mixing samples of feed mill A and ground corn of feed mill C reported higher initial pH value (0 hrs) for TPC (8.29) and BPW (7.24) respectively. Pellet loadout and wheat midds of feed mill A reported lower pH value for final TPC (6.31) and final BPW (5.15) respectively; ground corn of feed mill A reported higher pH value for final (24 hrs) TPC (7.39) and BPW (6.64).

Key Words: *C. perfringens*, *C. argentinense*, pre-enrichment

M44 Size and morphological variation among plasmacytes found during reactive plasmacytosis in lame ducklings Paul Cotter^{*1}, Ben Fetrow^{*2} *¹Cotter Laboratory, ²Maple Leaf Farms*

Reactive plasmacytosis exists when the proportion of bone marrow plasmacytes (PC) increases from far less than 1% to more than 2%. Morphological diversity accompanies increased frequency. The purpose is to

describe plasmacyte variation of bone marrow characteristic of reactive plasmacytosis. The method employed light microscopy of touch preparation smears obtained from femurs taken from commercial type lame ducklings ranging in age from 1 – 4 wk. Slides were prepared on farm site and later stained by Wright-Giemsa. The results. PC of typical size (area ~ 130 -150 μm^2) and shape (round to oval) are presented first. These are followed by variants including dwarf types with areas less than those common to erythrocytes (area ~ 75 μm^2). Others having irregular shapes (polygonal and rectangular) with serrated or hairy edges were found. Cytoplasmic staining differences ranging from deep purple “primitive” types to sky blue “derived” types characterized some of the variants. Some variant PC produce extracellular extensions of cytoplasm, a “net”, with the capacity to entrap bacteria whose presence is likely responsible for the reactive status of BM. This behavior is reminiscent of what has been described for heterophils. The association between giant granulated reticulum cells and nongranulated histiocytes with PC at various developmental stages is also considered. Atypical PC of delta shape and Mott cell variation will also be demonstrated. The latter are differentiated by variation of Russell body (RB) diameter ($<1\ \mu\text{m} - 3\ \mu\text{m}$) and homogeneity and the presence of nuclear immune globulin known as Dutcher bodies. Conclusions. It is presumed that any BM atypia cell has the potential to be found in the circulation complicating the interpretation of the hemogram. And so, demonstrating differences among plasmacytes participating in “reactive plasmacytosis” is an indication of the complexity of the anti-bacterial immune response. Furthermore, many atypical PC display similarity to those of multiple myeloma (MM) a malignant condition, and so these observations should be of interest to investigators of basic immunity.

Key Words: plasmacyte, bone marrow, atypical cells, lameness, duckling

SCAD

M46 Evaluation of an in-vitro inhibitory methodology utilizing field strains of *E. coli* and *Salmonella* spp. as assessment of probiotic effectiveness in poultry John Schleifer¹, Dorte Sandvang¹, Virginia Baxter², Charles Hofacre², Jean-Christophe Bodin¹, Stephanie Frankenbach¹, Christophe Bostvironnois¹ ¹*Chr. Hansen A/S*, ²*Southern Poultry Research Group*

As the dietary feeding of probiotics to commercial poultry continues to increase in North America the development of fast, reliable in-vitro methods to determine probiotic effectiveness is paramount. It is advantageous that the methods reflect in-vivo application standards and be easily interpreted by commercial technicians. An in-vitro method has been developed to visually assess the effectiveness of *Bacillus*-based probiotics against field isolates. The assay utilizes an agar well diffusion method and standard microbiological procedures. Visualization of the probiotic effectiveness can be determined within 48-72 hours. Over a period of 18 months, from February 1, 2020 to July 31, 2021, 57 different *E. coli* and *Salmonella* isolates were evaluated utilizing this procedure. Isolates were harvested from commercial poultry operations from five different US states. Samples were obtained from different commercial locations including poultry environmental areas, broilers, turkeys, and commercial layers. Single isolates of *E. coli* or *Salmonella* were cultured for the assessment. The isolates to be tested were selected by commercial production personnel based on clinical or informational importance. The probiotic used in the assay is a 3-strain *Bacillus*-based probiotic containing two *B. subtilis* and a *B. amyloliquefaciens* strain. An inhibitory effect on the growth of the *E. coli* or *Salmonella* spp. was observed in 56 of the 57 samples. The radii of inhibition ranged from 1mm to 3mm distant from the inoculation point of the probiotic. This methodology clearly demonstrates an inhibitory effect associated with the metabolic activity of the probiotic. The inhibitory effect observed using this methodology is rapidly visualized and efficiently communicates probable probiotic effectiveness.

Key Words: Bacillus, Probiotic, Inhibition, Assay, Pathogen

M47 Novel genes involved in the biofilm formation of Avian Pathogenic *Escherichia coli* Meaghan Young^{*GS}, Aline de Oliveira, Lisa Nolan, Catherine Logue, Nicolle Barbieri *University of Georgia*

Avian pathogenic *Escherichia coli* (APEC) is the etiological agent of avian colibacillosis, a leading cause of morbidity and mortality in the poultry industry worldwide. Although *E. coli* can be found as a harmless commensal organism in the gastrointestinal tract of most warm-blooded animals, APEC harbor specialized virulence factors distinguished from other *E. coli* strains that promote survival in or on the host and function in adhesion, invasion, and nutrient uptake. A key component of survival and persistence is through the formation of biofilms. Although much research has been conducted on *E. coli* biofilms, it is still unclear which genes are involved in the biofilm formation process of and are most prevalent in APEC. Therefore, the objective of this study was to identify novel genes involved in the biofilm formation ability of APEC. A total of 15,660 mutants of a well-characterized APEC serogroup O18, ST95 strain (APEC 380) were randomly created using the signature tagged mutagenesis technique and evaluated for decreased biofilm formation ability using the crystal violet assay. Mutants with a >50% decrease in biofilm formation ability compared to the wild type were sequenced around the transposon insertion and analyzed with BLAST-N for putative biofilm formation genes. A total of 547 putative biofilm formation genes were identified, including those

already known to be involved in biofilm formation along with those not known. To determine which genes were most important in APEC, 30 of the identified genes not known to be involved in biofilm formation were analyzed via PCR for prevalence in 109 APEC and 104 avian fecal *E. coli* (AFEC) isolates. A total of 9 genes had significantly higher prevalence ($p = 0.05$) in APEC than AFEC isolates. The selected genes are associated with several cellular processes, including lipopolysaccharide core biosynthesis and energy production, and some have unknown functions. The presence of these genes in APEC at a significantly greater rate than AFEC suggests that these genes are important in APEC biofilms and can be used as potential targets for antimicrobials and other therapeutics without disrupting commensal *E. coli*. Further research will evaluate the importance of these genes throughout different phases of biofilm production.

Key Words: Biofilm, *Escherichia coli*, APEC, Poultry

M48 Protective Efficacy of Diamond V XPC Diet against APEC Challenge in Poultry. Catherine Logue^{*1}, Anne Devorak¹, Breck Peterson¹, Yu-Yang Tsai¹, Meaghan Young¹, Hiliary Hsieh¹, Charlize Nakatsu¹, Nicolle Barbieri¹, Timothy Johnson² ¹*University of Georgia*, ²*Diamond V Mills*

Avian pathogenic *Escherichia coli* (APEC) is a significant cause of systemic extra-intestinal disease in poultry, clinically known as colibacillosis. APEC-associated disease results in significant morbidity, mortality and carcass condemnation at slaughter resulting in annual losses for the poultry industry. Live vaccines and probiotics are commonly used by producers to control bacterial infections; however, vaccines and probiotics may be ineffective against the diversity of disease-causing pathogens, including APEC. In this study, we assessed the use of Diamond Vs XPC feed containing a yeast byproduct as a gut health enhancer and its potential protective effect against an APEC challenge using the well-characterized highly pathogenic APEC O78 strain. In each of two identical trials, 120 chickens were split into eight groups: four groups were fed a control diet while the other four groups were fed the test diet of XPC containing the yeast by-product for 21 days. On day 14 of the study, birds were challenged with either Phosphate Buffered Saline (PBS) (control) or APEC O78 (intratracheally or orally) at a concentration of 1×10^8 cfu. On day 21, the birds were euthanized and necropsied. Swabs of heart blood and air sac and tissues of the liver, spleen, lung and ceca were aseptically collected for bacterial counts. Additionally, all birds were scored on necropsy for evidence of lesions consistent with colibacillosis in the liver, lungs, spleen, air sacs and heart using a standard rubric. APEC was detected in challenged birds fed the control diet at a similar rate to the supplemented feed for cecal counts however counts for other organs differed. In addition, lesion scores for challenged birds were consistently lower for birds on the XPC diet than the control suggesting that the yeast by-product had some level of protection against challenge. The route of challenge resulted in greater systemic disease and significantly higher lesion scores for intratracheally challenged birds compared to birds orally challenged and these scores were lower for birds on XPC than the control diet. This study provides valuable insight into the use of feed additives and their potential protective efficacy in poultry health.

Key Words: APEC, Poultry, challenge, yeast byproduct, protection

M49 Antimicrobial resistance and characterization of *Escherichia coli* isolated from poultry litter. Maryann Khong^{*1UG}, Shawna Weimer¹, Ashlyn Snyder¹, Anna Magnaterra¹, Nicolle Barbieri², Meaghan Young²
¹University of Maryland, ²College of Veterinary Medicine University of Georgia

Escherichia coli is present in the microbiota of both animals and humans, commensal, zoonotic, and has the potential to be pathogenic and can be a source of antibiotic resistance. The purpose of this study aimed to investigate the antibiotic resistance profile and antibiotic resistance, heavy metal, and minimal predictors of Avian Pathogenic *E. coli* (APEC-like) genes of *E. coli* isolates from poultry litter. *E. coli* was isolated (n = 68 isolates) from litter samples collected from 16 pens of broiler chickens on d53. Antibiotic resistance to ampicillin, azithromycin, colistin, cephalothin, imipenem, norfloxacin, sulphonamides, streptomycin, tetracycline, and trimethoprim/sulfamethoxazole were detected by performing the Kirby Bauer Test. *E. coli* isolates presented antibiotic resistance to cephalothin (52.3%), tetracycline (27.9%), streptomycin (27.9%), ampicillin (19.1%), colistin (13.2%), sulphonamides (11.8%), and imipenem (1.5%). Multi-drug resistance to at least three antibiotics was observed in 22.1% of isolates. The presence of 24 genes were assessed through PCR. To determine the likelihood of resistance when heavy metal and antibiotic resistance genes were present, a chi-square analysis was used. To determine whether the presence of one gene increases the likelihood that another gene is present, odds ratios were performed. There was a greater likelihood that genes tetA, groEL, aph(3)IA, silP, pcoD, sull, aadA, qacEdelta1, iron, ompT, and hlyF were present when there was ampicillin resistance (P≤0.05). There was also a greater likelihood that the gene groEL was present when isolates were resistant to ampicillin, colistin, tetracycline, sulphonamides, or cephalothin (P≤0.05). Genes that are minimal predictors of APEC-like (cvaC, iron, ompT, hlyF, etsB, iss, aerJ, ireA) isolates increased the likelihood of the presence of at least one heavy metal or antibiotic resistance gene (P≤0.05). Of all genes that associated the likelihood of another being present, 82.6% were minimal predictors of APEC-like isolates. Since antibiotic resistance is a threat to public health, consistent monitoring and research of *E. coli* are essential to developing effective solutions, improving management practices in the poultry industry, and furthering the One Health objective.

Key Words: *Escherichia coli*, antibiotic resistance, broiler, Kirby bauer, poultry litter

M50 Proventriculitis in a broiler company in Mississippi: a field investigation Gunnar Dunnam^{*GS}, Alejandro Banda *Mississippi State University Poultry Research and Diagnostic Lab*

This case report aims to describe the diagnostic approach involved in a field investigation of proventriculitis in broilers from an integrator in Mississippi. A broiler integrator had recent concerns over feed passage in the field as well as proventriculitis and decreased gizzard tone at posting sessions in one of the complexes. During a posting session of complex A and complex B, approximately 100 proventriculi/ventriculi were collected and processed for histopathology and fresh tissue for viral isolation and PCR testing. Grossly affected proventriculi during a separate posting session of complex B were also collected. This sampling represented 25 flocks. Testing on the collected samples included RT-PCR to detect chicken proventricular necrosis virus (CPNV), histopathology, and viral isolation. No samples were positive for CPNV. Histologically, proventriculitis ranged from mild to severe with 42 mild, 20 mild-moderate, 36 moderate, 2 moderate-marked, and 1 severe. Virus isolation revealed the presence of reovirus in 14 out of 25 flocks and adenovirus in 2/25. There was no statistical correlation between age of bird and degree of proventriculitis. Further statistical analysis of the correlation between complex and degree of proventriculitis will be completed. In addition, the correlation between degree of proventriculitis and virus isolation will be analyzed. Although the ultimate cause of proventriculitis in this integrator could not be ex-

plained by the diagnostic approaches applied, the potential role of reovirus in causing proventriculitis should be evaluated further.

Key Words: Proventriculitis

M51 Focal Duodenal Necrosis: identification of the potential role of Gram-negative bacteria in intestinal lesions Yu-Yang Tsai^{*1GS}, Catherine Logue¹, Nicolle Barbieri¹, Alvin Camus² ¹Poultry Diagnostic and Research Center, Department of Population Health, The University of Georgia, ²Department of Pathology, The University of Georgia

Focal Duodenal Necrosis (FDN) is an intestinal disease and considered one of the top five diseases in the table egg layer industry. However, the etiology has not been identified. Some research has identified *Clostridium colinum*, and *Clostridium perfringens* from FDN samples which might take part in the disease. In one paper, abundant detection of unknown Gram-negative filamentous bacteria was found within FDN lesions. In this research, we aimed to identify the Gram-negative filamentous bacteria commonly found in association with FDN lesions. A total of 59 ethanol fixed duodenum samples were collected from 8 different FDN affected farms. We observed FDN lesions in 42 duodenum samples. On gross lesion analysis, we found focal to multifocal erosions in duodenum tissues consistent with FDN. On microscopic analysis of the lesions, heterophilic and lymphoplasmacytic enteritis with loss of enterocytes at the villous tips, luminal fibrinonecrotic exudate, and variable numbers of filamentous bacteria were observed within the lesions. Laser Capture Microdissection was used followed by sequencing of the 16S rRNA gene to identify the Gram-negative filamentous bacteria found within the lesions. Preliminary results found that *E. coli* represents a large proportion of the microbial composition, followed by *Enterococcus faecalis* and *Acinetobacter spp.* An additional 20 fresh duodenum samples were collected from one FDN affected farm. Ten samples had FDN microscopic lesions. We isolated a total of 47 colonies, including 31 aerobic colonies and 16 anaerobic colonies from FDN samples on MacConkey agar and blood agar. Through 16S rRNA gene PCR and Sanger sequencing, we identified 39 colonies as *E. coli*, 3 identified as *Staphylococcus* and 5 were unidentified. Further virulence gene analysis by PCR found 70.2% of isolates examined possessed APEC virulence genes and 93.6% possessed Inflammatory Bowel Disease virulent determinants. The research shows that *E. coli* might be a contributor to FDN.

Key Words: Focal Duodenal Necrosis, Laser Capture Microdissection

M52 Retrospective analysis of an emerging *Enterococcus cecorum* outbreak in a Southern US broiler integration Gunnar Dunnam, Jay Thornton, Martha Pulido-Landinez^{*} *Mississippi State University, Poultry Research and Diagnostic Laboratory*

The goal of this retrospective study is to describe the clinical signs, lesions, and site of isolation observed in an *Enterococcus cecorum* outbreak experienced by a No Antibiotics Ever (NAE) vertical integration in the Southern US during 2021. Using a convenience sampling method, 86 cases received at the Poultry Research and Diagnostic Laboratory of Mississippi State University from January to October were analyzed. The peak in cases was observed from June to August. From the total number of cases, 82 (95.34%) were related to broilers chickens with an average age of 20.4 days. In breeders, 4 (4.65%) were observed with an average age of 30.3 weeks. Interestingly, this bacterium was isolated from more than one sampled site in the same group of chickens submitted for necropsy. Most of these cases were related to systemic disease and leg problems with isolation sites as follows: liver (n=34), pericardium (n=30), and legs (bone marrow, femoral head, tendon, and joint, n=95). The traditional presence of this bacterium in vertebrae was observed only in 3 cases. These findings suggest this agent is causing an important systemic disease in chickens with significant impact on legs. In broilers, coinfection with other agents was also analyzed, the most important being Avian reovirus (63.4%), fol-

lowed by bacteria (32.9%) including *Escherichia coli*, *Salmonella* spp. and *Gallibacterium anatis*.

The emergence of this *Enterococcus cecorum* systemic disease must be considered as important in terms of poultry health, and because it can negatively impact the productive performance of the affected chickens. It is possible the practice of NAE is contributing to the presentation of this novel systemic disease.

Key Words: *Enterococcus cecorum*, Emerging disease, leg problems

M53 Performance data in broilers challenged with *Eimeria* species after coccidial vaccination. Taylor Boyett^{*1GS}, Rocio Crespo¹, Daniel Adams^{1,2}, Kimberly Livingston¹ ¹North Carolina State University, ²University of Georgia

The *Eimeria* species are apicomplexan protozoa parasites that cause coccidiosis. In this study, we evaluated the performance of broilers challenged with *Eimeria* species after being vaccinated at day one with a commercial coccidial vaccine. A total of 720 one-day-old chickens were sexed and randomly distributed in 40 pens and raised to 35 days of age. The pens were arranged in a 2x2 factorial design, making a total of eight treatment groups. The two factors consisted of vaccination at day one, and *Eimeria* challenge at 21 days of age. Each group had 10 replicates. Live production, performance data, enumeration of coccidia oocysts by McMaster technique from fecal samples were evaluated. Performance results, feed conversion, coccidiosis evaluation, and coccidial counts will be discussed. Additionally, the intestines were weighed, examined and scored for the presence of gross lesions, and samples taken for RT-PCR and IL-10 ELISA assay that will be discussed in two associated presentations during this conference (Presented by Dr. Daniel Adams and Ms. Carissa Gaghan).

Key Words: feed additive, eimeria, coccidiosis, broiler

M54 A meta-analysis: microbiome of chickens infected with coccidia Andrea Pietruska^{*1GS}, Rüdiger Hauck^{1,2} ¹Department of Pathobiology, Auburn University, ²Department of Poultry Science, Auburn University

Coccidiosis causes considerable annual losses in the poultry industry, due to increased mortality, decreased performance in egg and meat production and expenses for treatment and prevention of the disease. Strict regulations, drug resistance, and changes in customer demand towards residue-free products and organic raised chickens made it necessary to look for new treatment strategies. The investigation of the microbiome of chickens is necessary to find alternative ways to improve the gut health of chickens and fight enteric diseases. In recent years, several studies were performed to investigate the influence of coccidiosis and treatments onto the microbiome of the chickens. Until now, no meta-analysis has been performed to synthesize the results of these experiments. The aim of our research was to summarize and analyze the data of these studies as basis for future research on the relationship of microbiome and coccidiosis. We performed a meta-analysis on raw data of five microbiome experiments on chickens. Each experiment contained a group inoculated with *Eimeria* spp. and a control group. All studies used 16SrRNA Illumina sequencing and the raw data were publicly available. For consistency, all raw data sets were reanalyzed independently in qiime2. The meta-analysis of the data set was performed in R using the SIAMCAT package. The results of the analysis of abundance of the phyla showed that *Bacteroidota* and *Firmicutes* were less abundant but *Proteobacteria* and *Desulfobacteriia* were enriched in the groups with coccidia infection compared to the control groups in most of the studies. Significant differences between the control group and the coccidia infected group were found in one study each for *Desulfobacterota*, *Proteobacteria*, *Firmicutes*, and *Enterococcaceae*, *Exiguobacteraceae*, *Bacillaceae*, and *Lactobacillaceae*. Variance plots showed a strong impact by differences between the variables study, 16s rRNA gene region, intestinal segment, and *Eimeria* species. In contrast, the infection status had only little influence on the genera. In conclusion, the heterogeneity of these microbiome studies makes recognizing common trends difficult.

Far more studies are needed to identify common changes of the bacterial microbiota after coccidia infection.

Key Words: microbiome, gut health, chicken, coccidiosis, meta-analysis

M55 Could anticoccidial sensitive *Eimeria* spp. recovered from wild turkey feces be a potential solution to control coccidiosis in commercial turkey operations? Carolina Trujillo Peralta^{*1GS}, Danielle Graham¹, Roberto Senas Cuesta¹, Callie Selby¹, Makenly Coles¹, Aaron Forga¹, Marcela Cardona¹, Lauren Lavery¹, Lucas Graham¹, Juan Latorre¹, Lisa Bielke², Guillermo Tellez-Isaias¹, John Barta³, Billy Hargis¹ ¹Department of Poultry Science, University of Arkansas, ²Department of Animal Sciences, The Ohio State University, ³Department of Veterinary Pathobiology, Ontario Veterinary College, University of Guelph

Although drug-sensitive live coccidiosis vaccines have been successfully used to control coccidiosis and renew drug sensitivity in commercial broiler and layer operations, limited species coverage vaccines are available for commercial turkey production. Previously, we obtained ~100 wild turkey fecal samples and isolated the 5 major *Eimeria* spp. relevant to commercial turkey operations. Preliminary studies were conducted with *Eimeria* spp. recovered from wild turkeys to confirm sensitivity to monensin, zonalene, and amprolium. The purpose of the present study was to assess the protective efficacy of an *E. meleagridis* vaccine candidate against homologous challenge. Experimental groups included: 1) Non-vaccinated, non-challenged control (NC); 2) Non-vaccinated, challenged control (PC); 3) candidate vaccine (VX) + amprolium; or 4) VX. For groups 3 and 4, 50% of the poult (directs) were orally vaccinated at DOH with 50 sporulated *E. meleagridis* oocysts and were comingled with non-vaccinated (contact) poult for the duration of the study. Group 3 was treated with amprolium (0.024%) in the drinking water from d10-14. At d23, all groups (except NC) were orally challenged with 95K *E. meleagridis* oocysts/mL/poult. The following parameters were evaluated to assess protective efficacy of VX +/- amprolium intervention: BW/BWG, intestinal lesion scores (LS) 6 days post-challenge, and daily fecal and litter oocysts per gram (OPG). Performance during the pre-challenge period was not impacted by VX. During the post-challenge period (d23-29), BWG for the VX groups was significantly ($P < 0.05$) higher than the PC group. LS were significantly ($P < 0.05$) reduced for both contacts and directs of VX groups compared to PC. As expected, amprolium administration from d10-14 markedly reduced fecal and litter OPG as compared to the group that did not receive amprolium. These data suggest that vaccination with this strain of *E. meleagridis*, with or without amprolium intervention, protected against homologous challenge without hindering performance. Future experiments will evaluate potential *E. dispersa*, *E. gallopavonis*, *E. adenoides*, and *E. meleagridis* vaccine candidates to control coccidiosis in commercial turkeys.

Key Words: *Eimeria*, coccidiosis, vaccine, turkeys

M56 A microbial physiology approach to the detection and enumeration of *Clostridium perfringens* in the poultry house environment Sydney Kinstler^{*1GS}, Margie Lee², John Maurer¹ ¹Virginia Tech, Department of Animal and Poultry Sciences, ²Virginia Tech, Biomedical Science and Pathobiology

Necrotic enteritis (NE) is a significant intestinal disorder of poultry caused by the spore-forming, obligate anaerobe, *Clostridium perfringens*. Antimicrobials have historically prevented NE until concerns over antibiotic resistance resulted in pressures to limit use in poultry, causing NE reemergence. *C. perfringens* spores persist in the environment, including poultry farms and potentially cause significant disease. Our central hypothesis is avian pathogenic *C. perfringens* strains produce high spore loads on some farms, predisposing birds to NE by spore ingestion from a heavily contaminated environment. Litter samples were collected in areas suspected of high spore loads in houses with varying histories of NE with suspicions of an outbreak. Capitalizing on *C. perfringens* physiology and metabolism,

we have developed a media formulation that “poisons” other poultry environmental bacteria using an oxidizing agent coupled with a physical heat treatment to determine spore abundance. The medium was formulated to be selective and differential for *C. perfringens* spores compared to other species in poultry litter that cannot survive conditions where nitrate reductase activity produces cytotoxic chlorite from a chlorate source, such as *C. perfringens* can. Lecithinase activity by *C. perfringens* is visible on egg yolk, a lecithin source that forms distinct white zones around the colonies. Coupled with a heat treatment to eliminate vegetative cell growth, spore isolation from poultry litter was achieved. Using our medium formulation and heat treatment, we identified lecithinase-positive colonies from the poultry house environment and subsequently confirmed them as *C. perfringens* by MALDI-TOF. We specifically found *C. perfringens* spores in the environment of poultry farms with a history of NE compared to farm without NE. The ability to monitor *C. perfringens* spore abundance is a game changer to understanding NE on poultry farms.

Key Words: necrotic enteritis, litter, *Clostridium perfringens*, media

M57 A flavonoid-rich corn cultivar decreases the incidence of necrotic enteritis in broilers Vinicius Buiatte^{*1GS}, Dorian Dominguez¹, Tyler Lesko¹, Mark Jenkins², Surinder Chopra¹, Alberto Gino Lorenzoni¹
¹The Pennsylvania State University, ²United States Department of Agriculture

Market restrictions and consumer preference for antibiotic-free products have led the poultry industry to move towards antibiotic-free production. The discontinued use of antimicrobial growth promoters (AGP) has been associated with an increased incidence of avian necrotic enteritis (NE). NE is an important disease that affects the intestinal tract of chickens, causing great economic losses to poultry producers. Therefore, alternatives to AGP

are needed. Flavonoids are plant-derived compounds known for their anti-inflammatory, antibacterial, and antifungal effects. A new corn cultivar developed at The Pennsylvania State University was genetically selected to contain high concentrations of flavonoids (PennHFD). We hypothesized that the inclusion of a flavonoid-rich corn in the diets of broiler chickens could reduce the incidence of NE. The purpose of our study was to evaluate the effects of dietary PennHFD on growth performance, incidence, and severity of intestinal lesions, in chickens challenged with NE. In a completely randomized design, 400 day-old chickens were allocated into 20 pens, and each pen received one of the following treatments: Control + Diet A (commercial corn line); Control + Diet B (PennHFD); Infected (co-infection of *Eimeria maxima* and *Clostridium perfringens*) + Diet A; Infected + Diet B. On day 13, chickens from the infected treatments were gavaged with 5,000 oocysts of *E. maxima*, followed by two in-feed inoculations (Days 18 and 19) of *C. perfringens* (1x10⁹ cfu/chicken). Chickens were raised for 21 days, and at the end of the experiment, data were collected to evaluate body weight gain (BWG), feed conversion ratio (FCR), mortality, incidence of intestinal lesions, and lesion scores. Infected chickens treated with PennHFD had 57% lower mortality ($P<0.05$), 52% lower incidence of intestinal lesions ($P<0.05$), 42.6% increase in BWG ($P<0.01$), and 7 points lower FCR ($P<0.01$), when compared to infected chickens treated with a commercial corn line. In conclusion, PennHFD was effective to reduce mortality and to improve growth performance of chickens undergoing NE. The inclusion of PennHFD in the diets of broilers undergoing necrotic enteritis may be a potential alternative for improving health and performance in chickens raised without antibiotics.

Key Words: Flavonoid, Necrotic Enteritis, Poultry, Phytogetic, Performance

Metabolism and Nutrition: General Nutrition

M58 Effects of Syngenta Enogen® corn on egg yolk color Hanna Alcocer^{*1GS}, Morgan Gravely¹, Dima White², Xiaoxing Xu¹, Daniela Alambarrio¹, Woo Kim², John Gonzalez¹
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The objective of this study was to evaluate the effects of Syngenta Enogen® corn on yolk color during laying. Leghorn hens ($N=320$, 18 wk, $n=80$ /treatment) were randomly allocated to 1 of 4 treatment groups: conventional poultry diet containing corn and soybean meal (PC), PC -200 kcal/kg ME (NC), PC replaced with Syngenta Enogen corn (PCS), and NC replaced with Syngenta Enogen corn (NCS). Eggs were collected every 5 wk from wk 25 to 45 for objective [spectrophotometer; L* (lightness) and b* (yellowness)] and subjective (visual panel; DSM Yolk Color Fan) measurement of yolk color. Data were analyzed as a completely randomized design with repeated measures. There were no Treatment×Week interactions for L* and b* ($P>0.76$), but there was a Week effect ($P<0.01$) for L*. Week-45 yolks had greater L* compared to other wk ($P<0.01$). Week-25, -35, and -40 yolks were not different from each other ($P>0.19$), but had greater L* values than wk-30 yolks ($P<0.01$). There were Treatment and Week effects for b* ($P<0.01$). Yolks from PCS and NCS did not differ ($P=0.55$) in b*, but had greater b* than PC and NC yolks ($P<0.01$). Yolks from PC and NC did not differ ($P=0.25$). Week-40 and -45 yolks had greater b* than all other wk ($P<0.01$) but did not differ ($P=0.52$) from each other. Week-25 yolks had greater b* than those from remaining wk ($P<0.01$), and wk-35 yolks had greater ($P<0.01$) b* than wk 30. There was a Treatment×Week interaction ($P<0.01$) for visual yellowness score. At wk 25 and 30, PCS and NCS yolks were not different ($P=0.12$) from each other, but had greater yellowness scores than PC and NC yolks ($P<0.01$), which were also not different ($P=0.33$). At wk 35, NCS yolks had greater ratings than all other treatments ($P<0.05$), while PCS yolks had

greater ratings than PC and NC yolks ($P<0.01$). Yolks from PC and NC treatments did not differ ($P=0.58$). At wk 40, NCS yolks had a greater ($P<0.01$) ratings than all other treatments, which were not different from each other ($P>0.31$). At wk 45, yolks from PCS and NCS were not different ($P=0.52$) from each other but had greater ratings than the PC and NC yolks ($P<0.01$). Yolks from NC treatment had greater ($P = 0.02$) ratings than PC yolks. Including Enogen® corn in layer diets increased objective and subjective yolk yellowness.

Key Words: diet, egg, layer, nutrition, visual panel

M59 The Effect of Feeding Sweet Potato and/or Peanuts on Layer Performance and Egg Quality Kari Harding^{*1GS}, Thein Vu², Rebecca Wysocky¹, Ramon Malheiros¹, Kenneth Anderson¹, Ondulla Toomer²
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Demand for high energy feed ingredients (i.e. corn) has increased within the poultry industry, human consumption and ethanol production in the US. This competition has increased feed cost, making it crucial for the poultry industry to find economic alternative and effective feed ingredients. Past research indicated that variations of sweet potato product could be an acceptable ingredient in poultry feeds. Our research aim was to examine the effect of high oleic peanut (HOPN) and by-products (SWP) on hen production and egg quality. Five isocaloric, isonitrogenous dietary treatment were used in this study, a conventional control (C1), an soy protein-isolate control (C2), 4% SWP diet (T1), an 8% HOPN (T2), and a 4% HOPN 4% SWP (T3). Seven hundred twenty, Shaver laying hens were randomly assigned to one of the five treatments, with four replicates of thirty-six birds per treatment. Body and feed weights were recorded bi-weekly. Eggs were tabulated daily and totaled bi-weekly. Thirty eggs

per treatment replicate were collected bi-weekly for egg quality analysis and 16 eggs per treatment were used for chemical analysis. All data was analyzed using a 1-way analysis of variance at $P < 0.05$ significance. There were no treatment differences in egg or body weights. Hens fed the C1 and C2 diets produced a significantly more ($P < 0.05$) total dozen of eggs compared to T3 SWP HOPN. Birds fed T2 diet consumed more total feed/bird than C1 and T3 diets. The feed conversion ratio (kg/doz.) for the T1, T2, and T3 were higher ($P < 0.05$) than C1 and 2. Shell thickness, Haugh Unit, and albumen height were greater in eggs produced from hens fed the C1, T1, and C2 treatments at wk 2. Yolk color was significantly darker in eggs produced from hens fed T1 relative to the others at wk 2. Yolk color was significantly darker in eggs produced from hen fed the C1 and T1 diets compared to T2 eggs at wk4 and wk 6. There were no differences in the percent large USDA grade A eggs constituting the primary size and grade. While there were no differences in hen performance with feeding SWP and/or HOPN, the results suggest that egg yolk color may be enhanced with feeding laying hens a SWP supplement diet.

Key Words: alternative feed ingredient, sweet potato, laying hen, egg quality, high-oleic peanuts

M60 Effects of soybean meal processing conditions on growth performance and intestinal integrity in coccidia vaccinated broiler chickens Ana Villegas^{1GS}, Anita Menconi², Nadia Yacoubi², Brian Jordan¹, Todd Applegate¹ ¹*Department of Poultry Science, University of Georgia*, ²*Evonik Operations GmbH, Nutrition & Care*

Soybean meal (SBM) is an ingredient with a high digestibility of protein, Lys, and Met. An optimal heating process of SBM is required to eliminate antinutritional components that negatively interfere with digestion, absorption, and metabolism of nutrients. The effect of 3 commercial SBM batches were subjected to different heat treatments to evaluate its effect on growth performance and intestinal integrity. A total of 1,860 male Cobb 500 broiler chicks were vaccinated at 1d of age with a commercial coccidia vaccine (Coccivac®-B52) via coarse spray. The 3 SBM batches were processed with different extruder temperatures of 182, 199, and 154 °C for normal-control, overcooked, and undercooked SBM, respectively. Increasing processing temperature ensured reduction of trypsin inhibitor, protein dispersibility index, and KOH solubility. Each treatment was fed to 10 pens of 62 birds from 0 to 35d of age. SBM was fed as a fixed inclusion in the diet, which notably SBM varied by CP by 49.2, 47.9, and 46.1 % amongst the control, overcooked, and undercooked SBM, respectively. Body weight gain (BWG), feed intake (FI), and feed-to-gain ratio (FCR) were recorded on d 0, 14, 28, and 35. The relative weights of the right pectoralis major (RPM) were determined from 5 birds per pen at 35d of age. The jejunal and ileal region of the small intestine was evaluated to calculate the villus to crypt length ratio on d14. Serum FITC-D concentrations was assessed in 1 bird per pen for gut permeability at d 16. Intestinal permeability increased in birds fed the overcooked SBM ($P < 0.05$). On d 14, 28, and 35, overcooked SBM depressed BWG, FI, and increased FCR ($P < 0.05$). The undercooked SBM decreased the villus height to crypt depth ratio in the jejunum on d14 and reduced BWG from 0-14 and 0-35d periods compared to the SBM control group. The relative weights of the RPM decreased significantly in 35 d-old birds fed overcooked SBM ($P < 0.05$). The adverse effects of overcooked SBM on BWG were driven by digestible amino acid (A.A) intake, which was lower ($P < 0.05$) for Lys, Met+Cys, and Thr in the overcooked treatment compared to the normal and the undercooked treatment groups. In conclusion, improper heat treatment of SBM affects bird performance and the intestinal integrity of broiler chickens.

Key Words: Soybean meal, Heat treatment, Trypsin inhibitor, Protein dispersibility index, KOH solubility

M62 Energy values of faba beans and field peas for broiler chickens using a regression method Abidemi Adekoya^{*GS}, Olayiwola Adeola *Purdue University*

Faba beans (FB) and field peas (FP) are legume seeds that serve as sources of starch and protein and have different cultivars with varied nutrient composition. Hence, an accurate evaluation of gross energy (GE) of FB and FP is necessary for precision nutrition with the least cost formulation. There-fore, 2 experiments (Exp) were conducted to determine the ileal digestible energy (IDE), metabolizable energy (ME), and nitrogen-corrected ME (MEn) of organic FB and Ds-Admiral FP (FPD) (Exp. 1), and Hampton FP (FPH) and 4010 FP (FP4) (Exp. 2) by regression analysis. A total of 240 birds were allotted to 5 diets in a randomized complete block design with BW as a blocking factor on d 18 and 17 post hatching in Exp. 1 and 2, respectively. There were 8 replicate cages with 8 birds per cage. Test ingredients were added to a corn-soybean meal-based reference diet at 150 or 300 g/kg by replacing the energy-contributing ingredients. On d 23 and d 22 in Exp. 1 and 2 respectively, birds were euthanized by CO₂ asphyxiation and dissected for ileal digesta collection. In Exp. 1, the ad-dition of FB or FPD to the reference diet linearly decreased ($P < 0.01$) the apparent ileal digestibility (AID) of GE, and IDE in diets. There was a quadratic response ($P < 0.05$) on apparent total tract utilization (ATTU) of GE, ME and MEn with increasing concentration of FB. Inclusion of FPD to the reference diet linearly decreased ($P < 0.01$) the ATTU of GE,

ME and MEn. The IDE, ME, and MEn determined were 2,541, 2,628, and 2,426 kcal/kg DM in FB and 2,254, 2,540, and 2,358 kcal/kg DM in FPD, respectively. In Exp. 2, graded concentration of FPH or FP4 in the reference diet linearly decreased ($P < 0.01$) ATTU of GE. A linear decrease ($P < 0.001$) was detected in ME, and MEn of diets with inclusion of FPH. The AID of GE, and IDE were linearly decreased ($P < 0.01$), while the ME and MEn in diets were quadratically decreased ($P < 0.01$) as the concentration of FP4 increased. The IDE, ME, and MEn determined by regression were 3,274, 3,033, and 2,872 kcal/kg DM in FPH and 3,019, 3,155, 3,009 kcal/kg DM in FP4, respectively. In conclusion, the energy values in faba beans and field peas demonstrated in the current study may be used in diet formulation.

Key Words: faba beans, field peas, energy, broiler, regression

M63 Energy utilization of hydrolyzed feather meal and flash-dried poultry protein for broiler chickens Opeadura Osunbami^{GS}, Olayiwola Adeola *Purdue University*

Two experiments were conducted to determine the ileal digestible energy (IDE), metabolizable energy (ME), and nitrogen-corrected ME (MEn) concentrations of 2 poultry by-products, which included hydrolyzed feather meal (HFM) and flash-dried poultry protein (FDPP) for broiler chickens. In experiments (Exp.) 1 and 2, HFM or FDPP were incorporated into a reference diet at 3 levels (0, 75, or 150 g/kg) by replacing the energy-yielding ingredients. In both experiments, dry fat was utilized as a lipid source and titanium dioxide was included at 5 g/kg as an indigestible marker in the diets. All diets in both experiments were formulated to maintain the ratio of corn to soybean meal to dry fat at 12.6:6.4:1. Each diet was allocated to 8 replicate cages with 6 birds per cage in a randomized complete block design. Body weight was the blocking factor. In Exp. 1, the inclusion of HFM, linearly decreased ($P < 0.05$) the MEn although, the ME concentration in the diets were linearly increased ($P < 0.05$). In Exp. 2, a linear increase ($P < 0.05$) was observed on the DM ileal digestibility while ileal digestibility of energy was linearly decreased ($P < 0.05$). The total tract retention (TTR) of DM and energy linearly increased ($P < 0.05$) with the inclusion of FDPP. Similarly, as the dietary concentration of FDPP increased, ME and MEn concentration was also observed to linearly increase ($P < 0.05$). In broiler chickens, the respective regression-derived IDE, ME, and MEn evaluated for HFM in Exp. 1 were 4,509, 4,250, and 3,745 kcal/kg DM with corresponding values of 3,221, 4,710, and 4,081 kcal/kg DM for FDPP in Exp. 2. This study provides useful information on the energy concentrations in HFM and FDPP for their formulation into the diets of broiler chickens.

Key Words: Broiler chickens, by-products, digestibility, flash-dried poultry protein, hydrolyzed feather meal

M64 Estimation of individual feed intake of broiler chickens in group-housing systems Jung Yeol Sung^{GS}, Olayiwola Adeola *Purdue University*

Broiler chickens are reared in group-housing systems such as cages or pens in experimental situations. When mortality of birds occurs during the experiment or birds are identified as outliers, estimation of individual feed intake (IFI) of birds is required to adjust pen feed intake for IFI of the birds in interest. The objective of this study was to compare averaging, ratio, and partitioning methods of estimating the IFI of broiler chickens in group-housing systems. The averaging method, often referred to as Bird-day method, assumes that the amount of feed consumed by each bird in a cage is equal regardless of its body weight (BW). In contrast to the averaging method, the ratio method considers IFI of a bird as proportional to its BW gain. In the partitioning method, IFI is calculated as the sum of IFI for maintenance and growth. To validate these methods, 32 male broiler chickens (initial BW = 161 ± 19 g) at day 7 post hatching were individually housed in cages. Birds had a free access to a corn-soybean meal-based diet for 28 days. Body weight and feed disappearance were

recorded weekly, and excreta were collected over the last 3 days of each week. Collected excreta were used to determine the ME in the diet, which was required for the partitioning method. Growth performance data of 32 birds were used to create 8 artificial cages with 4 birds or 4 artificial cages with 8 birds for either a completely randomized design (CRD) or randomized complete block design (RCBD). Estimated IFI was calculated for the 3 methods with the actual IFI and BW gain of 4 or 8 birds within cages, and the difference (%) between actual and estimated IFI was calculated for all birds. Data of cages with 4 and 8 birds were pooled together for statistical analysis. The respective difference between actual and estimated IFI for CRD or RCBD were lower ($P < 0.05$) at 4.5% each for the partitioning method compared with the averaging method at 14.9 or 14.1%, and the ratio method at 7.1 or 7.2% from days 7 to 35. In conclusion, the partitioning method accurately estimated IFI of broiler chickens in experimental situations with group-housing systems.

Key Words: broiler, energy for maintenance, feed intake estimation, growth, metabolizable energy

M65 New methodology to measure internal particle size in pellets and to quantify the degree of grinding during pelleting Susan Bonilla^{GS}, Joseph Gulizia, Santiago Sasia, Martha Rueda, Wilmer Pacheco *Department of Poultry Science, Auburn University*

Grinding is one of the highest cost centers during feed manufacturing and it is typically accomplished using hammermills or roller mills. In pelleted diets, the pelleting process leads to additional grinding, which can lead to inconclusive results regarding optimum particle size in broiler diets. The aim of this study was to develop a new methodology to evaluate average particle size of pellets after particle agglomeration and determine the degree of grinding that occurs during pelleting. Corn was ground using a two-pair roller mill to obtain 3 corn particle sizes (801, 1587 and 2394 μm). Ingredients were blended using a twin shaft mixer to produce the mash diets, which were steam conditioned at 77°C with a retention time of 45 seconds and pelleted through a 4.0 mm pellet die. A total of 36 mash and pellet samples were collected at even intervals from a 1-ton batch for each corn particle size. Twelve mash samples were analyzed as collected using the ASABE method S319.4 while 12 mash and 12 pellet samples went through an additional procedure of hydration and dehydration using a novel methodology to separate agglomerated particle after pelleting. The objective of hydration and dehydration of mash diets prior to pelleting was to ensure that the new methodology did not alter particle size or particle size distribution. The experiment consisted of a factorial arrangement of 3 corn particle sizes and 3 feed forms (meal, hydrated meal, and pellet). Data were statistically evaluated using the GLM procedure of JMP and means were separated by Tukey's HSD with a statistical significance considered at $P < 0.05$. In diets with corn particle size of 801 μm and 1587 μm , meal and hydrated meal had higher ($P < 0.05$) particle size than the internal particle size within pellets (914 and 963 vs 716 μm) and (1311 and 1352 vs 779 μm) respectively. In diets with corn particle size of 2,394 μm , hydrated meal had higher ($P < 0.05$) particle size compared to the particle size within pellets (1678 vs 1085 μm) with the meal having an intermediate particle size of 1590 μm . This new methodology helped to quantify the degree of grinding that occurs during pelleting, which could help the poultry industry to accurately determine the optimum particle size requirements of broilers.

Key Words: Pellet, roller mill, feed manufacture, particle size

M66 Effect of corn samples from different origins on broiler performance, processing yield and nutrient digestibility from 1 to 35 days of age. Jose Vargas Patino^{GS}, Joseph Gulizia, Susan Bonilla, Santiago Sasia, Wilmer Pacheco *Auburn University*

This study was conducted to determine the effect of feeding corn from 3 origins (USA, Argentina, and Brazil) on broiler performance, processing yield and nutrient digestibility from 1 to 35 d of age. A total of 900 d-old

Ross 708 × YPM male broilers were randomly sorted in 36 floor pens each containing 25 chicks and subjected to 3 experimental diets with 12 replicates per treatment. Experimental corn-soybean based starter, grower and finisher diets were formulated to meet or exceed the Ross 708 broiler nutrient specification guidelines, being only different in corn origin (USA, Argentina, and Brazil). Feed intake, BW, BWG, and FCR corrected for mortality were determined at 10, 21 and 35 d of age. At 35 d of age, ileal digesta was collected from 4 birds per pen for nutrient digestibility analysis, and 10 birds per pen were processed for assessment of processing yield. All collected data were analyzed using the GLM procedure of JMP, and Tukey's HSD was used for mean separation with statistical significance considered at $P \leq 0.05$. No significant differences ($P > 0.05$) were found on BW, BWG and feed intake from 1 to 35 d of age among the treatments. However, broilers fed diets with USA and Brazilian corn had lower FCR in comparison to the broilers fed diets with Argentinian corn from 1 to 35 d of age (1.434 and 1.434 vs 1.452 g/g; $P \leq 0.05$). Broilers fed diets with Brazilian corn had a higher breast weight compared to broilers fed diets containing Argentinian corn (575 vs 553 g; $P \leq 0.05$), but did not differ from the breast weight from broilers fed diets with US corn (575 vs 556 g; $P > 0.05$). Corn origin did not influence ($P > 0.05$) crude protein and fat digestibility. However, broilers fed diets with corn from USA and Brazil had greater phosphorus (P) (63.37, 62.23 vs 55.26%; $P \leq 0.05$), calcium (Ca) (41.59, 43.85 vs 30.23%; $P \leq 0.05$), and potassium (K) (88.98, 87.97 vs 86.04%; $P \leq 0.05$) digestibility compared to broilers fed diets with corn from Argentina. Overall, corn origin influenced growth performance, breast weight, and P, Ca, and K digestibility of broilers from 1 to 35 days of age.

Key Words: corn origin, broilers, ileal digestibility

M67 Effects of breeder flock age (pullet or late) and feeding strategy (choice or no choice) as it influences 0-14 d bird beak capacity, feed particle selection, and 0-14 d performance Maria Alvarenga¹GS, Pratima Adhikari¹, Joseph Moritz², Kelley Wamsley¹ ¹Mississippi State University, ²West Virginia University

Early literature suggests that broiler feed particle size (FPS) preference may be linked to bird beak capacity (BBC); however, this theory has yet to be confirmed. Research in our lab showed that 0-14 d broilers can consume crumbles up to 3736 μm without negatively impacting starter and 0-46 d performance. This study utilized a 2 Breeder Flock Age (BFA) x 2 Feeding Strategy (FS; choice or no choice) factorial arrangement to determine the relationship between BBC, complete FPS preference and subsequent 0-14 d performance. The BFA utilized were Pullet (27 \pm 1 wk; PBFA) and Late (51 \pm 1 wk; LBFA) to provide two beginning chick sizes. A common feed was manufactured into pellets and crumbles. A common crumble (~1050-1200 μm) was fed to no choice (NC; NCFPS), remaining feed was sieved/placed into 1 of 10 FPS classifications for choice FPS (CFPS) pens (ranging from pan-4750 μm). Ross x Ross 708 males of both BFA (n=800) were obtained from the same hatchery. At d 0, chicks were individually weighed (by BFA) and placed into 18 weight classes/BFA; a chick/weight class was randomly assigned/pen. Each pen (n=40) had 10 numbered feeders arranged around the pen wall. Daily feeder order (consequently feed) was randomly assigned; each feeder had daily feed intake (FI) measured. For NCFPS, a common crumble was provided/feeder; for CFPS, 1 of 10 FPS was offered/feeder. For BBC, 8 birds/pen were tagged for beak measurements using digital calipers (L, W, H, opening) on every other d for 14 d. As expected, 0-14 d BW for PBFA was lower than LBFA ($P < 0.05$); similarly, FI was lower ($P = 0.0215$). In general, smaller BBC measurements were obtained for PBFA chicks vs. LBFA ($P < 0.05$). Also, 0-14 d FI, FCR and BW were not influenced by FS ($P > 0.05$). Daily FPS placed was consistently higher for CFPS vs. NCFPS ($P < 0.05$); FPS consumed (FPSCON) was higher for NCFPS at d 2, 4-9 and higher for CFPS on d 11 and 12 ($P < 0.05$). No difference in FPSCON was found for d 1, 3, 10, 13 and 14 ($P > 0.05$). Notably, FPSCON was not affected by BFA ($P > 0.05$). On average, FPSCON was ~1700 and 2200 μm at 0-7 d (CFPS

and NCFPS) and ~2200 μm at 7-14 d (regardless of FS). Data suggest that while BFA influences 0-14 d performance and BBC, it may not impact the FPSCON; these data may be used to determine optimal starter FPS.

Key Words: Broiler, Beak capacity, Feed particle size, Feed selection, Performance

M68 Histological and gene expression analyses of broiler jejunal and liver tissues from commercial farms in relation to flock performance.

1. Histological findings Davis Fenster¹GS, Candice Blue¹, Don Ritter², Luis Romero³, Rami Dalloul¹ ¹University of Georgia, ²Poultry Business Solutions LLC, ³anh-innovation Lda.

Four flocks of straight run Ross-708 broilers of the same age were selected from four farms with a wide variation of recent historic FCR in a commercial US broiler integration. Flocks were on a NAE, all-vegetable diet program and received a coccidiosis vaccine at hatch. At 28 d, a total of 16 healthy, randomly selected birds per farm were weighed and euthanized. At necropsy, coccidiosis lesions were visually scored (1-4) and evidence of necrotic enteritis was recorded. Further, a cross section sample of 1 cm of jejunum, 1 cm posterior to Meckel's diverticulum, was collected for histological analysis. Histology slides were examined blindly using a set of 13 criteria that included coccidiosis lesions, villus length, crypt depth, intraepithelial lymphocytes, heterophils infiltration, dysbacteriosis, and goblet cells, amongst others. At slaughter (63 d), final BW, FCR, and mortality were calculated for all 4 flocks, and for each of the 3 houses of Farm 1. Normally distributed data were analyzed with the lme package of R and multiple comparisons used the Sidak adjustment and a $P < 0.05$ significance level. Final FCR were 1.951, 1.901, 1.914, and 1.923 and final BW were 4.188, 4.246, 4.309, 4.096 kg/bird for Farms 1 to 4, respectively. No evidence of intestinal lesions due to *E. tenella* or *E. acervulina* was found at necropsy. Only 3 birds from Farm 3 and 1 bird from Farm 4 presented *E. maxima* lesions in the jejunum. No difference in average daily gain at sampling was evident. Nonetheless, significant differences in jejunal crypt depth, villi length: crypt depth ratio, heterophils counts, cystic crypts, and intraepithelial lymphocytes among farms were detected, with Farms 2 and 3 generally presenting healthier tissues. Crypt depths were 392, 280, 262, and 376 μm (SEM = 21.9) for Farms 1 to 4, respectively. Crypt depth, villi length: crypt depth ratio, and heterophil counts were highly correlated with performance at 63 d ($|r| > 0.90$), whereas heterophil counts were negatively correlated with ADG ($r < -0.95$). Histology analyses at 28 d of age demonstrated differences correlated to broiler performance, which were not evident from macroscopic observation during necropsy. A further study evaluated differences in gene expression amongst these broiler flocks.

Key Words: broiler, histology, jejunum, performance, gene expression

M69 Effect of starter diet nutrient restriction and feed form on broiler chickens until 19 days of age Joshua Flees¹GS, Caroline Gregg, Brittany Wall, Gerardo Abascal-Ponciano, Charles Starkey, Jessica Starkey ¹Auburn University Department of Poultry Science

Discovering the etiology of Wooden Breast has been the focus of many research studies. Previous work aimed at slowing broiler growth in with starter diet nutrient restriction revealed broilers could overcome a 10% targeted reduction in ME and dLys by increasing their feed consumption, thus, maintaining similar BW. Further diet restrictions have not been evaluated for creating a model for slowing BW gain (BWG) to reliably generate both Wooden Breast-affected and unaffected broilers. The objective of this randomized complete block design experiment with a 2x3 factorial treatment structure was to evaluate the effect of the combination of starter diet nutrient restriction and feed form on broiler growth performance. Ross 708 × Yield Plus male broilers (n = 360) were reared for 19 d on new litter in raised floor pens (n = 5 birds per pen). Birds were fed 1 of 2 corn and soybean meal-based diets: a standard industry starter diet as the control (CON) or a starter diet with a 30% targeted reduction in ME, dMet, and dLys (RES). Each CON and RES diet was offered in 1 of 3 feed

forms: crumble (CRUM), meal (MEAL), or reground crumble (REGR) equivalent to the MEAL particle size. Birds remained on the same diet and feed form for the entire 19 d and were weighed on d 3, 7, 10, 13, 16, and 19. Mortality-corrected feed intake, BWG, and feed conversion ratio were determined. Data were analyzed as a 2-way ANOVA with the GLIMMIX procedure of SAS. Means were separated using the PDIF option at $P \leq 0.05$. On d 3 and 7, birds fed MEAL diets were lighter than birds fed CRUM or REGR diets ($P < 0.0001$). Birds fed RES diets were lighter than CON fed birds on d 7 ($P = 0.0062$). From d 10 to 13, birds fed MEAL form CON and RES diets had lower BW ($P < 0.0268$) among all treatments while RES MEAL-fed birds had the lowest BW from d 16 to 19 ($P < 0.0178$). From d 0 to 19, birds fed RES REGR diets consumed the most feed ($P < 0.0001$) were the least efficient ($P < 0.0001$) and gained less weight when fed RES diets ($P < 0.0001$). When CRUM were fed, BWG was higher compared with MEAL or REGR feed forms ($P < 0.0001$). Starter diet restrictions and MEAL feed forms successfully lowered broiler BW and slowed BWG and contribute to the establishment of a model to study Wooden Breast myopathy development.

Key Words: diet nutrient restriction, diet feed form, amino acid reduction, metabolizable energy reduction, growth performance

M70 The effect of oil type and oil quality on the fatty acid profile of the liver and adipose tissues in 20-day-old broiler chickens Richard Adefoye^{*1GS}, Merlin Lindemann¹, Anthony Pescatore¹, Michael Azain², Michael Ford¹, Sunday Adedokun¹ ¹University of Kentucky, ²University of Georgia

The objective of this study was to determine the effect of oil type and oil quality on the fatty acid (FA) profile of the liver and adipose tissues (abdominal and subcutaneous) in 20-day-old broiler chickens. The experiment used 192 day-old male by-product Cobb breeder chicks in a randomized complete block design with 4 treatments consisting of 8 replicates with 6 birds per replicate, in a factorial arrangement of treatments involving 2 oil types (corn oil [CO] vs. soy oil [SO]) and 2 oil quality levels (fresh CO; peroxide value [PV] = 3 meqO₂/kg and fresh SO; PV = 4 meqO₂/kg vs. oxidized CO; PV = 104 meqO₂/kg and oxidized SO; PV = 109 meqO₂/kg). The birds were fed corn-SBM-based diets for 20 days. Data were analyzed using the appropriate GLM procedure of SAS. The locational distribution of the essential fatty acids (EFAs; C18:2n-6 and C18:3n-3) and the most abundant FAs (C16:0, C18:0, and C18:1) were compared across the liver, abdominal fat, and subcutaneous fat. In both adipose tissues, the most abundant FA was C18:1 (oleic acid), followed by C18:2n-6 (linoleic acid), regardless of dietary treatment. Conversely, in all diets, C18:2 n-6 was the most abundant followed by C18:1. The comparison between the liver and adipose tissues showed that the abdominal fat had a higher ($P < 0.05$) unsaturated fatty acid (UFA) level, while the liver had a higher ($P < 0.05$) level of saturated fatty acids (SFAs), polyunsaturated fatty acids (PUFAs) and SFA:UFA. Oil type and oil quality independently influenced the abundance of the EFAs in the liver, as SO resulted in higher ($P < 0.05$) content of C18:2n-6 and C18:3n-3 compared with CO, while oxidized oil reduced the contents of C18:2n-6 and C18:3n-3 compared with fresh oil. In the abdominal and subcutaneous fats, the SO group had a higher ($P < 0.05$) level of C18:3n-3, and a lower level of C18:3n-6 (gamma-linolenic acid), compared with the CO group. Interestingly, birds that received diets containing fresh CO had a higher ($P < 0.05$) content of C18:2n-6 in their abdominal and subcutaneous fats compared with those that received fresh SO. Based on these results, there is an active post-absorptive modification of FAs. Furthermore, both oil quality and oil type influenced the modification of EFAs in the adipose tissues and liver.

Key Words: oxidized oil, Oil quality, broilers, oil type, soy oil

M71 Validation of a previously determined nitrogen-corrected metabolizable energy value for distillers dried grains with solubles on more recently collected samples from 4 different biorefineries Benjamin Parsons^{*1GS}, Kyle Teague¹, Kenia Mitre¹, Kevin Herrick², Melissa Jolly-Breithaupt², Samuel Rochell¹ ¹Center of Excellence for Poultry Science, University of Arkansas System Division of Agriculture, ²POET Bioproducts

This experiment was conducted to validate a previously determined average MEn value of 2,765 kcal/kg (DM) for distillers dried grain with solubles (DDGS) sourced from 11 different biorefineries using similar processing conditions. A growth assay in which the dietary energy intake of birds was quantitatively and qualitatively restricted was used. Dietary treatments included: 1) a corn and soybean meal based diet fed ad libitum, 2) a reduced energy basal (REB) diet restricted fed to achieve approximately 60% of the feed intake of treatment 1, 3) a restrict-fed REB diet with soybean oil (REB + SO) added (1.44% to provide 125 kcal/kg) at the expense of cellulose, and 4 to 7) restrict-fed diets containing 1 of 4 DDGS sources included at 15% and formulated to be isocaloric to the REB based on a value of 2,765 kcal MEn per kg for each DDGS (DM). A common diet was fed until 14 d post-hatch when treatments were administered to 9 replicate cages (6 chicks/cage) of male chicks after BW were equalized among groups. Nutrient content of the restrict-fed REB diet was increased to ensure that energy was the first limiting component for growth. Body weight gain, feed intake, and feed conversion ratio (FCR) were measured from 14 to 21 d, though BW was the direct driver of FCR given the equalized intake across all restrict-fed groups. Statistical analyses were based on a one-way ANOVA and means separation by a Tukey's test. Birds fed the REB + SO diet had a 4.8% higher BWG ($P < 0.05$) and a 7.2 point lower FCR ($P < 0.05$) than birds fed the REB diet. Birds fed the REB + DDGS 2, 3, 4, 5 all had BWG that were similar ($P > 0.05$) to those fed the REB diet and lower than that of birds fed the REB + SO. The BWG of birds fed DDGS 1 was intermediate to that of birds fed the REB and REB + SO diets. Feed conversion ratio was lowest for birds fed REB + SO, highest for birds fed REB and DDGS 3 and 4, and intermediate for birds fed DDGS 1 and 2 ($P < 0.05$). Thus, these data indicate that dietary energy was the first limiting component for growth when restrict-feeding the REB and that inclusion of 15% DDGS from 4 different sources using a value of 2,765 kcal MEn/kg (DM) led to similar performance as birds fed the REB, validating this energy value across all sources.

Key Words: metabolizable energy, DDGS, regression

M72 Comparison of the Ark NE and AMEn values of soybean meal and corn for fast-growing male broilers from d0 to d56 Nawin Suesattajit^{*GS}, Jordan Weil, Cole Umberson, Pramir Maharjan, Diego Martinez, Craig Coon ¹University of Arkansas, ²Center of Excellence for Poultry Science

Arkansas Net Energy (Ark NE) is a NE system for ingredients and diets that provides actual productive energy and is dependent upon genetics, age, environment and nutrient content. The Ark NE values for complete diets and ingredients are based on performance, body composition and heat production providing net energy for maintenance (NEm) and net energy for gain (NEg). Broilers need a dietary energy system that reflects performance and high protein gains. The Ark NE and AMEn values of SBM and Corn were determined for starter, grower, and finisher I, II, and III feeding periods using dual energy x-ray absorptiometry (DEXA) and indirect calorimetry. Experiment 1 consisted of feeding three different levels of SBM in a corn-soybean meal basal diet in each feeding period. Experiment 2 consisted of feeding three levels of test corn substituted against a corn-soybean meal summit diet for each feeding phase. Data were subjected to a one-way ANOVA and Tukey's HSD to compare separate treatment means with significance set at 0.05 using JMP pro 15.2. A linear regression model was used to establish the three energy values of SBM and corn for broilers d0-d56 of age. The Ark NE and AMEn values (kcal/kg diet or ingredient) were significantly increased ($P < 0.05$) for broilers fed the highest concentration of protein and digestible amino acids. Overall

weighted values based on feed intake for 56d period for SBM Ark NE and AMEn were 3,028 and 2,342 kcal/kg. The respective Ark NE and AMEn weighted values of corn based on feed intake for 56d were 1,368 and 3,311 kcal/kg. Consequently, SBM weighted value for all feeding periods contributes 34% of the Ark NE for broiler diets, whereas SBM provides 21 % of AMEn calories for same diets. In contrast, corn contributes 40% of the Ark NE for diets, while it provides 77% of AMEn calories from same diets. Dietary Ark NE provides additional kcal for broilers from ingredients and diets providing digestible amino acids for lean mass gain (NEg) and fasting heat production (NEm).

Key Words: Soybean meal, Corn, Ark NE, DEXA, Indirect calorimetry

M73 Comparison between an in-line implementation of a temperature profile probe-based tool vs. three non-contact methodologies to monitor hot pellet temperature in broiler diets Andrea Rubio^{GS}, Sharon Chuah, Corey Wishon, Rachael Wood, Adam Fahrenholz *North Carolina State University*

Previous research has demonstrated that the heat generated during the pelleting process has an impact on the thermostability of feed additives, hence decreasing benefits on animal growth performance. Even though conditioning temperatures are obtained in real-time, the frictional heat generated by the die can only be estimated using non-contact methodologies. Therefore, the objective of these experiments was to evaluate the current available methodologies compared to a novel in-line methodology to estimate the rise in hot pellet temperature (HPT) as they are extruded by the die. Experiment 1 consisted of a broiler starter diet with 7 treatments, varying moisture contents from mixer added water (0, 1, and 2%) and conditioner added steam (0, 2, 4%). A mash diet not subjected to mixer added water nor conditioner added steam was dry pelleted to serve as negative control (NC). Three non-contact methodologies were used to collect 189 HPT readings at die exit: 1) insulated thermos, 2) Styrofoam bucket, and 3) infrared (IR) gun. Data were statistically evaluated using ANOVA and means were separated by Tukey's honestly significant difference. Experiment 2 constituted a 2×2×3 factorial arrangement of 2 fat inclusion levels (1 and 4%), 2 die L/D ratios (6.5 and 10), and 3 conditioning temperatures (165°, 175°, and 185° F). A temperature profile probe with 8 sensing points located within the pelleting chamber was used to collect 660 HPT readings at the die surface in real-time. Data were statistically evaluated as a 2×2×3 (fat level × die compression × conditioning temperature) randomized factorial arrangement. In experiment 1, the lowest HPT was obtained when the sample was analyzed with the IR gun ($P < 0.05$). The temperature readings obtained with the IR gun in these studies fluctuated at ambient temperature which allowed heat to escape within the pellets, leading to an underestimation of HPT. In experiment 2, interactions were apparent ($P < 0.05$) for all treatments. Diets pelleted with a thicker die, 1% fat level, and conditioned to 185° F had the highest HPT readings ($P < 0.05$). The results of these experiments indicated that HPT readings differed depending on the methodology used.

Key Words: broiler, die, hot pellet, probe, thermostability

M74 Assessment of the quality of extruded full fat soybean meal subjected to different processing temperatures Danny Patino^{GS}, Michael Joseph *North Carolina State University*

Although there are different types of protein used today, soybean meals (SBM) are the most widely used source of high-quality vegetable protein for livestock, aquaculture, and especially for poultry feed. It may be processed in a variety of different ways to increase the protein concentrations and to deactivate anti-nutritional factors, where this feed ingredient is exposed to varying degrees of heating during its processing. However, overheating can damage protein and reduce nutritional value. This study was conducted to determine the quality of full-fat soybean meal (FFSBM) exposed to different processing temperatures. Common lab tests including urease index (UI), crude protein (CP), KOH protein solubility (PS), pro-

tein dispersibility index (PDI), and trypsin inhibitor (TI) were measured as an indicator of over-processing and under-processing of FFSBM using the extrusion process. A single screw dry was used to produce 6 experimental FFSBM using die temperatures of 135°C, 145°C, 155°C, 160°C, 165°C, and 170°C in a commercial feed mill. These conditions were selected to achieve both under and overcooking of the soy protein, given the limitations of the equipment. UI, TI, PS, PDI, and CP of the 6 FFSBM produced during the extrusion process plus the raw soybeans were analyzed. Data were analyzed using one-way ANOVA and means were separated using Tukey's test. The UI in excess of a 0.15 increase in pH units suggests under processing, the content of UI of the raw beans was 2.09. However, after the extrusion process UI decreased less than 0.15 for all the FFSBM produced. The TI levels at 170°C were found to be the least at 0.49 mg/g and were statistically different from the other FFSBMs. The PS and PDI values suggested overprocessing at temperatures of 165°C and 170°C. The PS of the FFSBM produced at these temperatures has less than 65% suggesting overprocessing. However, for the FFSBM produced at temperatures lower than 165°C, there was no statistical difference. The PDI values decreased with an increase in temperature and CP increased from 37.59% in raw beans to 45.23% at 170°C with a consistent increase as the processing temperature was raised. Overall, the study showed that 160°C was the best operating temperature to get the best extruded FFSBM.

Key Words: soybean meal, extrusion process, under processing, over processing

M75 Understanding variability of soybean sucrose, oligosaccharide, and NSP content Muhammad Ali^{1GS}, Maria Alfaro-Wisaquillo¹, Gustavo Quintana-Ospina¹, Klaus Englyst², Gilson Gomes³, Edgar Oviedo-Rondón¹ ¹*Prestage Department of Poultry Science, North Carolina State University, 2**Englyst Carbohydrates Ltd, 3**AB Vista Inc*

Sucrose is the main soluble sugar in soybeans, and it is highly related to meal energy value. Soybean oligosaccharides and the non-starch polysaccharides (NSP) are poorly digested by poultry. Understanding variability of these compounds in soybeans can help improve their nutritional quality. In this study, the content of sucrose, raffinose, stachyose, total, soluble and insoluble NSP, as well as its relationship with CP, AA, and trypsin inhibitor (TI), were evaluated. The sources of variation were 4 commonly grown soybean varieties (A to D) planted in 3 North Carolina counties (X, Y, Z) in 2 maturity groups double crop (growing two successive crops per year) and full season (growing one traditional crop per year). 24 treatments resulted from a 4 x 3 x 2 factorial arrangement of treatments. A completely randomized design with four plot replicates was used. Raw soybean samples were cleaned from foreign material and standardized to be USDA grade 3 or 2. NSP content, including solubility fractions, was determined by GC analysis of constituent sugars, while sugars and oligosaccharides were determined by ion chromatography. Other nutrients (CP, AA, and TI) were determined by AOAC standard methods. The data was standardized to dry matter and later analyzed using a three-way ANOVA and mean separation was done by Tukey's test. Correlation analyses were conducted between carbohydrates and CP, AA, and TI content. Three-way interaction effects ($P < 0.01$) were detected in all parameters evaluated. Soybeans grown in double-crop with varieties A, C, and D in location Z had the highest sucrose. tNSP and raffinose content were higher in varieties A and D in location X regardless of maturity group. Variety C had the highest iNSP in location Y in both maturity groups. Variety C also had the lowest stachyose independently of location or maturity group. Location X and Z had lower CP and AA than Y. Negative correlations ($P < 0.001$) were detected between CP and total sugars ($r = -0.74$) and sucrose ($r = -0.71$). In contrast, TI resulted in positive correlations ($P < 0.001$) with total sugars ($r = 0.53$) and sucrose ($r = 0.51$). In conclusion, soybean variety, planting location, and maturity group affected soybean carbohydrate profile and their relationship with CP, AA, and TI.

Key Words: Soybean, carbohydrates, trypsin inhibitor, maturity season

M76 Effects of corn kernel hardness and drying temperature on nutrient composition, quality parameters, and early broiler performance. Joaquin Cabanas-Ojeda^{*1GS}, Nicolas Mejia-Abaunza², Paula Lozano-Cruz², Valmiro Lima Aragão Neto¹, Muhammad Ali¹, Maria Camila Alfaro-Wisaquillo¹, Gustavo Quintana-Ospina¹, Lina Penuela-Sierra², Gilson Gomez³, Edgar Oviedo-Rondon¹ ¹*North Carolina State University*, ²*Facultad de Medicina Veterinaria y Zootecnia, Universidad del Tolima*, ³*AB Vista*

Corn kernel hardness and post-harvest drying temperatures have been reported to affect the nutrient composition and corn nutritional quality. Young chickens have an immature digestive system, and performance can be affected by nutrient digestibility. This study evaluated how two corn varieties of different kernel hardness dried at a high or low temperature affect corn nutrient quality and early live performance. The hard and soft endosperm corn varieties were planted under similar conditions. Each variety was split into two groups at harvest to be dried at 120°C and 35°C, cleaned and ground in a hammer mill. Four treatments resulted from a 2x2 factorial arrangement with two corn kernel hardness and two drying temperatures. 15 samples per treatment were scanned in NIRS, and the spectra were analyzed using AB Vista calibration curves. A total of 960 Ross 708 male day-old chicks were placed into 48-floor pens and fed a starter diet within each treatment up to 14 d. Data were analyzed in a randomized complete block design. Results of nutrient composition indicated interaction effects for all parameters evaluated except PSI and vitreousness. NIRS results showed that corn with soft endosperm and any corn dried at 35°C had more ($P<0.001$) AME, PSI, fat, and fiber than the harder kernel variety or when drying at 120°C. However, higher protein and amino acid values were found in the hard variety and when dried at 120°C. Main effects were detected ($P<0.001$) for PSI and vitreousness. Higher PSI was observed in the soft variety, and when drying at 120°C, the PSI decreased. In contrast, drying at 120°C increased vitreousness in both corn varieties. Total and insoluble NSP were higher in the soft kernel corn ($P<0.001$), but drying at 120°C decreased all these contents in the soft variety. However, the A:X ratio was higher for hard endosperm and when drying at 120°C ($P<0.001$). In chicken performance, BW, BW gain, and FI were independently decreased by the hard endosperm variety and drying at 120°C. The adjusted FCR was better for corn dried at 35°C (1.17 g:g) than 120°C (1.19 g:g). The adjusted FCR was correlated with PSI ($r=0.36$) and vitreousness ($r=0.33$). In conclusion, PSI and vitreousness were more important in corn nutritional quality than nutrient or NSP composition.

Key Words: Corn, kernel hardness, drying temperature, NIRS, broiler

M77 Effects of corn kernel hardness and drying temperature on corn mean particle size and distribution post-grinding in a hammer mill Valmiro Lima Aragao Neto^{*1UG}, Paula Lozano-Cruz¹, Nicolas Mejia-Abaunza¹, Joaquin Cabanas¹, Muhammad Ali¹, Maria Camila Alfaro-Wisaquillo¹, Gustavo A. Quintana-Ospina¹, Lina Maria Penuela-Sierra¹, Virginie Riviere², Edgar Oviedo-Rondon¹ ¹*Prestage Department of Poultry Science, North Carolina State University*, ²*AB Vista*

The importance of particle size (PS) of ground corn on gut health and broiler performance is well accepted, with effects attributed to better gizzard development. Previous studies have shown that corn kernel hardness and post-harvest drying temperatures (TEM) affected PS and distribution. This study evaluated how two corn varieties with different kernel hardness dried at 120°C or 35°C affected geometric mean (D_{gw}) and standard deviation (S_{gw}) of PS and distribution by sieve pans. Two corn varieties of hard (H) and soft (S) endosperm planted under similar agronomic conditions, dried in an air-forced dryer, cleaned, and ground in a hammer mill in four opportunities was evaluated with five reps per corn treatment combination. The D_{gw} and S_{gw} were determined by the sieving method ASAE S319.3. Data was analyzed in a 2x2 factorial arrangement in an RCBD with grinding as a block considered a random effect in a mixed model. Interaction effects were observed ($P<0.001$) for D_{gw} and distribution of particles by pan. Drying at 120 °C increased the D_{gw} of the S corn

variety from 660 to 715 μ m and reduced D_{gw} from the H from 743 to 699 μ m. Main effects were observed on S_{gw} ($P<0.001$). Hard endosperm corn (3.51) was more uniform than the S (3.77). Drying at 120 °C reduced variability (3.58) compared to drying at 35 °C (3.70). Looking at particle distribution by sieve pan, interaction effects were evident ($P<0.001$). Soft endosperm kernels dried at 35 °C had 18.5% of particles <150 μ m and 120 °C TEM reduced it to 16.3%. While H kernels only had 14.8% particles <150 μ m independently of TEM. Drying the H kernels at 120 °C increased the proportion of particles between 150 and 1190 μ m (42%) compared to TEM 35 °C (36%). Drying at 120 °C decreased the proportion of particles >1191 μ m in H endosperm corn (43.3%) compared to drying at 35 °C (48.4%). TEM did not affect ($P>0.05$) the proportion of particles retained in pans with sieves >150 μ m for soft corn. In conclusion, kernel endosperm hardness and TEM affected D_{gw} . The S_{gw} of H endosperm was smaller, and drying at 120 °C reduced it. The higher TEM reduced the fine particles in S endosperm corn (<150 μ m) and the coarse ones (>1191 μ m) in the H endosperm, increasing the proportion of particles between 150 and 1190 μ m.

Key Words: Corn, kernel hardness, particle size, drying temperature, hammer mill

M78 Nutrient composition differences between whole corn kernels, broken kernels, and corn residues. Nicolas Mejia-Abaunza^{*1UG}, Joaquin A. Cabanas-Ojeda¹, Paula Lozano-Cruz¹, Valmiro Lima Aragao Neto¹, Muhammad Ali¹, Maria Alfaro-Wisaquillo¹, Gustavo A. Quintana-Ospina¹, Lina M. Penuela-Sierra¹, Virginie Rivera², Edgar O. Oviedo-Rondon¹ ¹*Prestage Department of Poultry Science, North Carolina State University*, ²*AB Vista*

Corn is the major ingredient in poultry feed in many regions of the world, and its quality and nutritional composition impact both animal performance and economics. Corn kernel hardness and post-harvest drying temperatures have been observed to affect the nutrient composition. It has also been reported a lower nutritional value of broken corn kernels and residues. This study evaluated the nutritional differences between corn kernels, broken kernels, and corn residues of two corn varieties of different kernel hardness dried at a high or low temperature. Two corn varieties of hard and soft endosperm were planted under similar agronomic conditions. At harvest, each variety was split into two groups to be dried at 120°C and 35°C. All corn treatments were cleaned in a seed-cleaner machine to separate corn kernels from residues and impurities. Furthermore, kernels were manually sieved to segregate impurities from broken kernels. Fifteen samples per treatment were scanned in NIRS, and the spectra were analyzed using AB Vista calibration curves. Data were analyzed in a 2 x 2 x 3 factorial arrangement of treatments with kernel hardness, drying temperature, and corn cleanliness as the main effects. NIRS results of nutrient composition indicated three-way interaction effects ($P<0.001$) for all nutrients evaluated. Higher AME was observed for intact corn kernels (clean) than broken kernels and corn residues (175 kcal/Kg maximum difference). Corn residues presented lower ($P<0.001$) protein solubility index (PSI) regardless of corn variety or drying temperature, and broken kernels presented intermediate values at every level of particle size. Similarly, starch values were lower in residues except for the soft variety dried at 35°C. As expected, vitreousness was higher for the hard corn variety in all corn cleanliness levels ($P<0.001$). The hard corn variety and drying at 120°C presented higher ($P<0.001$) protein values than their counterparts. In conclusion, cleaning corn improved the AME and PSI of the two corn varieties dried at a high or low temperature. Sieving residues to obtain broken kernels improved content of some nutrients evaluated on the corn by-product, obtaining under some conditions, similar nutrient values to the ones observed in the whole clean corn.

Key Words: Corn, kernel hardness, NIRS, hammer mill, cleanliness

M79 Nutrient composition of corn particle fractions generated during hammer milling Paula Lozano-Cruz^{*1UG}, Nicolas Mejia-Abaunza¹, Valmiro Lima Aragão Neto¹, Joaquin A. Cabanas-Ojeda¹, Gilson A. Gomes², Virginie Rivera², Edgar O. Oviedo-Rondon¹ ¹*Prestage Department of Poultry Science, North Carolina State University*; ²*AB Vista*

The particle size of ground corn has been an important feed factor related to intestinal health, broiler performance, and environmental sustainability. These benefits have been mainly related to better gizzard development. Previous studies have shown that corn kernel hardness and post-harvest drying temperatures affected nutrient composition values and particle size. We hypothesize that fractions of corn particles differ not only in physical properties but also in nutrient composition. Two corn varieties of different endosperm hardness (hard and soft) were planted with the same agronomic conditions, harvested and dried at 120 °C or 35 °C, cleaned in a seed cleaner, and ground in three opportunities in a hammer mill. Seven different particle size ranges were obtained by the sieving method ASAE S319.3 (0-52, 53-149, 150-296, 297-840, 841-1190, 1191-1679, and 1680-more microns). The contents of each sieve pan were retained, accumulated from five replicates per treatment combination, and analyzed

by NIRS to obtain data of moisture, PSI, vitreousness, ash, AME, starch, protein, and AA using AB Vista calibration curves. Data was analyzed in a 2 x 2 x 7 factorial arrangement of treatments in a completely randomized design. Interaction effects were observed ($P < 0.001$) for all corn traits. A reduction in AME and PSI was observed as particle size reduced independently of the kernel hardness and drying temperature while starch and moisture were increased. The content of protein, Lys, Met, and fat was higher in the middle particle size groups (297 to 1679 μm). Hard kernel corn had higher AME, protein, Lys, Met, and vitreousness than soft endosperm corn. Drying at 120 °C generally reduced AME, fat, PSI, and ash compared to drying at 35 °C on both corn varieties. Fat was not affected ($P > 0.05$) by corn hardness, and starch was not affected ($P > 0.05$) by drying temperature. In conclusion, the nutritional content varies among the different corn particle sizes generated during grinding. The medium particle sizes seem to have the best balance among all nutrients to support broiler nutrition. Corn traits that produce great dispersion in their grinding may affect the live performance of poultry fed mash diets due to variability in nutrient content.

Key Words: Corn, Particle size, NIRS

Metabolism and Nutrition: Vitamins and Minerals

M80 Copper requirements of broilers fed phytase Patricia de Carvalho^{*1GS}, Sergio Luiz Vieira¹, Julmar Feijo¹, Heitor Rios¹, Walter Altevogt¹, Bernardo Barros e Xavier¹, Carolina Nunes¹, Pablo Ibarro¹, Douglas Drebes¹, Raquel Horn¹, Catarina Stefanello², Liris Kindlein¹ ¹*Federal University of Rio Grande do Sul*; ²*Federal University of Santa Maria*

Phytic acid is strongly negatively charged at physiological pH values indicating a tremendous potential for complexing positively charged molecules, such as copper (Cu). The objective of the present study was to reassess Cu requirements for broilers fed diets supplemented with phytase (Hiphos®, DSM) at a concentration known to break most of phosphate groups out of phytate (2,500 FTU per kg). A total of 280 d-old Cobb male broilers were randomly distributed in battery cages (0.9 x 0.4 m²) and assigned to 5 treatments with 8 replicates per treatment. A basal corn-soybean meal-based control diet was formulated (8.3 ppm analyzed Cu). Four additional treatments contained 3, 6, 9, and 12 ppm of added Cu from sulfate pentahydrate (CuSO₄5H₂O). On d 28, all birds were euthanized and kidney, liver, tibia, *Pectoralis major* muscles, gastrocnemius tendons, excreta and ileal contents were collected. Data were analyzed using the SAS MIXED proc and means were separated using Tukey test with statistical significance considered $P \leq 0.05$. The analyzed Cu levels in the experimental diets were 8.3, 11.4, 13.8, 16.6 and 19.9 ppm Cu with corresponding 2752, 3200, 2831, 2446, 2947 FTU per kg. Dietary Cu did not influence ($P > 0.05$) Ht, Hb, locomotor disorders, Cu breast content as well as in tendons. However, FI and BWG from 8 – 28 d had quadratic responses with maximum responses estimated at 13.6 and 14.6 ppm; $R^2=0.110$ and 0.123 , respectively. Liver Cu increased linearly ($P < 0.05$, $R^2=0.352$) as the dietary level of Cu increased, whereas a quadratic response was observed for tibia Cu concentration ($P < 0.05$) with a maxima estimated at 22 ppm, $R^2 = 0.861$. Data from the present study suggest that dietary supplementation of Cu for broilers from 1 to 28 d of age is needed in average lower amounts than what is commercially used when fed 2,500 FTU per kg.

Key Words: growth performance, copper, micro-mineral, requirement, poultry

M81 Effect of partial replacement of inorganic zinc with zinc methionine and partial replacement of inorganic zinc/manganese methionine on live performance and processing yield Caleb Marshall^{*1GS}, Timothy Biggs², Donald McIntyre², Kelley Wamsley¹ ¹*Department of Poultry Science, Mississippi State University*; ²*Global Animal Products, Inc.*

Two concurrent studies were conducted to evaluate the effect of partial replacement of inorganic Zn (IZ) with chelated Zn (CZ; *ZinMet*®) or partial replacement of inorganic Zn and Mn with chelated Zn and Mn (CZM; *ZinMet*® and *ManMet*®, respectively), on male broiler performance and processing. Ross x Ross 708 male broilers (800/experiment; EXP) were randomly distributed to 32 pens (25 birds/pen; 2 treatments/EXP; 16 replications/treatment). Diets (corn and soybean meal based) were formulated to breeder recommendations and similar in nutrient composition, including digestible Met to account for the Met contribution of CZ and Mn. For EXP 1, diets were formulated to 100ppm of Zn; 100ppm IZ and with 40ppm of IZ partially replaced with CZ. For EXP 2, diets were formulated to 100ppm of Zn and Mn; 100ppm IZ + 100ppm inorganic Mn (IZM) and with 40ppm of IZ and Mn partially replaced with CZ + chelated Mn (CZM). On d 14, birds were orally gavaged with 20x dose of a live coccidiosis vaccine. In EXP 1, there were no differences for BW, BW gain (BWG), FCR, or FI for 0-14, 0-28, 0-42, and 0-56 d ($P > 0.05$). Although not significant, birds fed CZ had a numerically lower FI ($P=0.0512$), 100g less than IZ birds. For EXP 2, CZM increased 14 d BW, BWG, and FI ($P < 0.05$) compared to birds fed IZM; this trend continued for 28 d BW and BWG ($P=0.0978$; 0.1114 , respectively), though no benefit was found >28 d ($P > 0.05$). Birds fed CZM had no difference in FCR for 0-14, 0-28, 0-42, and 0-56d ($P > 0.05$). At 42 and 56 d, 320 birds were selected for processing 5 birds/pen $\pm 125\text{g}$ (42 d) and $\pm 200\text{g}$ (56 d) and processed on 43 and 57 d. In EXP 1, birds selected from IZ had higher 43 d Live Wt. (LW) and Leg Wt., as well as 57 d LW and Carcass Wt. ($P < 0.05$); though 43 and 57 d Breast Wt. and Yield were not impacted ($P > 0.05$). In EXP 2, CZM had no effect on 43 d LW, Breast Wt./Yield, and Carcass Wt. ($P > 0.05$), though birds fed CZM had higher Leg Wt. and Yield ($P < 0.05$) than IZM birds. At 57 d, IZM increased LW, Carcass, Fat Pad, and Leg Wts. when compared to birds fed CZM ($P < 0.05$). Data demonstrate that partial replacement of IZM with CZM improved 14 d BW and BWG only (EXP 2), and partial replacement of IZ with CZ (EXP 1) and IZM with CZM (EXP 2) had no impact on breast meat weight or yield.

Key Words: trace minerals, chelated minerals, broiler performance, zinc methionine, manganese methionine

M82 Effect of different dietary vitamin D3 levels on performance and bone quality in broilers Tainá Lopes^{*GS}, Mariana Vasconcelos, Isadora Araújo, Lorena Sousa, Bruno Costa, Leonardo Lara, Itallo Araújo *Universidade Federal de Minas Gerais*

An experiment was conducted to evaluate the effects of cholecalciferol (VitD3) levels in the diet of broilers from 1 to 35 days old, on performance and bone quality. A total of 360 male 1-old-day Cob500® broiler chickens were distributed in a completely randomized design, with 5 treatments with 6 replicates of 12 broilers each. Dietary treatments were: 1) 100%: 2,500 IU/kg starter/2,000 IU/kg grower. 2) 75%: 1,875 IU/kg starter/1,500 IU/kg grower. 3) 1,250 IU/kg starter/1,000 IU/kg grower. 4) 625 IU/kg starter/500 IU/kg grower. 5) 0 IU/kg in both phases. The data obtained were submitted to quadratic, exponential, and linear response regression models ($P < 0.05$), and the best adjustment obtained was analyzed using R software (2016). There was an effect of VitD3 levels on performance. Positive linear effect upon feed intake until the level of 26.53% at 14 days ($P < 0.001$; $R^2 = 0.65$), 25.52% at 21 days ($P < 0.001$; $R^2 = 0.95$) and 24.98% at 35 days old ($P < 0.001$; $R^2 = 0.98$). Similar linear effect was observed for body weight until the dose of 25.83% ($P < 0.001$; $R^2 = 0.74$), 25.88% ($P < 0.001$; $R^2 = 0.96$) and, 24.90% ($P < 0.001$; $R^2 = 0.99$) for 14, 21 and, 35 days old. Feed conversion ratio showed a positive linear effect until the dose of 23.88% ($P < 0.001$; $R^2 = 0.31$), 25.32% ($P < 0.001$; $R^2 = 0.77$) and, 24.76% ($P < 0.001$; $R^2 = 0.88$) for 14, 21 and, 35 days old. For bone parameters, the tibial bone ash showed a positive linear effect until the dose of 26.08% ($P < 0.001$; $R^2 = 0.88$), 28.85% ($P < 0.001$; $R^2 = 0.71$) for 21 and 35 days old. For calcium percentage it was a positive linear effect until the dose of 19.26 % ($P = 0.023$; $R^2 = 0.68$), 48.81% ($P < 0.001$; $R^2 = 0.26$) for 21 and 35 days old. The phosphorus percentage showed a positive linear effect until the dose of 19.12% ($P < 0.001$; $R^2 = 0.66$), 39.76% ($P < 0.001$; $R^2 = 0.46$) for 21 and 35 days old. The VitD3 supplementation allowed an increase in the bone-breaking strength rate of 0.57% up to 21 days reaching 21.31% of the VitD3 dosage and an increase of 0.91% up to 35 days reaching the dosage of 27.03%. The results showed here allow us to conclude that the inclusion of 625 IU and 500 IU of VitD3/kg of feed for the initial and growth phase guarantees the broiler's maximum performance and bone quality.

Key Words: cholecalciferol, performance, bone strength

M83 Evaluation of increasing additions of choline chloride on modern broiler chicken growth performance and carcass characteristics Caroline Gregg^{*IGS}, Zack Lowman², Kari Estes², Charles Starkey¹, Jessica Starkey¹ *¹Auburn University Department of Poultry Science, ²Balchem Corporation*

Choline is a crucial nutrient for broilers and as it acts as a precursor for cell signaling molecules and membrane phospholipids. The interconnected biosynthesis pathways of choline and methionine suggest that methionine can substitute for choline during a deficiency. Yet little research focusing on choline supplementation of current high-yielding broilers is available. The objective was to evaluate the effect of increasing additions of choline chloride on performance and carcass characteristics of broilers fed diets with reduced methionine and reared under summer environmental conditions. Ross 708 x Yield Plus male broilers were reared for 41 d on used litter in floor pens ($n = 31$ birds per 2.3 m² pen). Birds were fed 1 of 6 corn and soybean meal-based, reduced methionine diets containing 0, 400, 800, 1,200, 1,600, or 2,000 mg added choline chloride per kg of feed ($n = 12$ pens per treatment). Diets were provided in 4 phases: crumbled starter from d 0 to 15 with 0.42% dMet, pelleted grower from d 16 to 28 with 0.42% dMet, and pelleted finisher from d 29 to 41 with 0.37% dMet. Rearing temperatures began at 34.4 °C, were reduced gradually over 21 d to 26.7 °C and maintained there until d 41. Mortality-corrected feed intake (FI), BW gain (BWG), and feed conversion ratio (FCR) were determined. On d 43, 720 birds ($n = 10$ median birds per pen) were processed, and carcass part weights were determined. Carcass part yields were calculated as proportions of the cold carcass weight. Wooden Breast (WB) severity

was determined based on the proportion of the fillet affected by abnormal hardness. Data were analyzed as a 1-way ANOVA with the GLIMMIX procedure of SAS. Means were separated using the PDIFF option at $P \leq 0.05$. Supplemental dietary choline inclusion resulted in similar BWG ($P = 0.7408$), altered FI ($P = 0.0217$), and reduced FCR ($P = 0.0017$) from d 0 to 41. Supplementation of 1,200 mg per kg of feed or more linearly increased both breast and carcass yields ($P < 0.0001$). However, the incidence of severely affected WB fillets also increased with choline concentration ($P = 0.0004$). These results indicate that supplementing broilers during summer rearing conditions with dietary choline chloride may improve feed efficiency as well as increase carcass and breast yields.

Key Words: broiler chicken, choline chloride, growth performance, carcass yield, wooden breast

M84 Commercial dietary chelated organic trace mineral inclusion strategies on broiler breeder hen reproductive performance, egg quality and broiler offspring characteristics Luis Avila^{*IGS}, Kelly Sweeney¹, Melanie Roux², Bob Buresh², Dima White¹, Woo Kim¹, Jeanna Wilson¹ *¹University of Georgia Department of Poultry Science, ²Novus International*

Proper management and optimizing nutrition are some of the future challenges regarding broiler breeder hen production. Previous experiments demonstrate positive benefits of dietary chelated organic trace minerals in poultry. The objectives of this experiment were to evaluate the effect of including commercial blends of methionine hydroxy-analogue chelated trace minerals in broiler breeder hen diets on hen performance, egg quality and progeny quality. Cobb 700 pullets ($n = 1,540$) were reared in floor pens up to 21 wk of age and fed a common starter diet through 3 wk of age. Birds were then fed a common grower diet from 4 to 21 wk using a restricted skip-a-day feeding program. Pullets were then moved to laying pens ($n = 43$ per pen; $n = 10$ pens per treatment) with 4 Cobb Vantage males in each pen. Treatment diets were fed daily after 25 wk of age and were formulated to be iso-caloric, iso-nitrogenous and iso-methionine. Three treatment diets provided Zn, Mn, and Cu (mg per kg): CTL = predominantly inorganic trace minerals (143 -180 - 123); ORG = chelated organic trace minerals (ORG: 50 - 60 -15); and MIX = a blend of inorganic/chelated organic trace minerals (75/25 - 90/30 - 22.5/7.5). Data were analyzed using a GLM procedure and significance of $P \leq 0.05$ was used. Weekly hen BW, uniformity and egg weights were not consistently different through wk 65. However, MIX-diet maximized hen egg production after wk 39 ($P \geq 0.003$) and overall mean egg weight compared to those from CTL-fed hens (64 vs. 63 g; $P = 0.038$). Egg shell quality and cuticle deposition were improved when hens were fed MIX-diet in the second half of the laying period ($P \geq 0.009$ and $P = 0.019$, respectively). Flock fertility and hatchability remained unaffected by dietary trace mineral inclusion ($P \geq 0.141$ and $P \geq 0.205$, respectively). Even with broiler growth performance from the 4 hatches being similar, maternal ORG dietary supplementation increased hatched and 7-d-old chick bone mineral density ($P \geq 0.044$). These results indicate that combining inorganic and chelated organic trace minerals in broiler breeder hen diet is advantageous for sustaining high quality settable egg production. While feeding a chelated organic source of trace minerals improved chick bone density.

Key Words: broiler breeder hen, chelated-organic trace minerals, reproduction, hatchery, egg quality

Metabolism and Nutrition: General Nutrition

M85 Corn fermented protein a sustainable, alternative, functional protein for poultry from bioethanol biorefineries Peter Williams* *Fuid Quip Technologies*

Corn Fermented Protein (CFP) is a designed corn grain based, high concentration, fermented, functional protein concentrate derived by mechanical processing (Maximized Stillage Co-Product™ process) of the whole stillage stream of a dry grind ethanol plant. The product is regulatory compliant and complies with descriptions in the AAFCO handbook. From 8 ethanol plants in production, estimated USA production in 2022 is approximately 500 ktons per annum rising to one million tons in 22/23. The product has a 52% crude protein content and approximately 24% of the product dry matter is spent yeast. Benign drying maintains lysine availability >95%. In the ceacectomized rooster assay digestibility of 22 amino acids is 89.5% (n=10; SD 0.015). Both between different production plants and within a plant over time the product is highly consistent. The product has been extensively tested in ration formulations for all classes of livestock, aquaculture, and companion animals. The product is an excellent replacement for protein concentrates including soy and corn pro-

tein concentrates. Trials in broilers and turkey poulters when CFP was used up to 12.5% inclusion rates in commercial formulations to replace soya and corn protein concentrates demonstrated that compared with a control formulation, formulated according to the breed standards, there was no significant difference in weight gain or feed conversion efficiency up to 35 days of age, although the weight gain over the period was numerically greater when CFP was used as an alternative protein. When a range of protein replacement scenarios were considered, the carbon intensity score of a bioethanol plant running the MSC process is >20% which is the threshold set by EPA for being regarded eligible as a renewable fuel. In the European situation with diets formulated with CFP compared with imported soy bean meal the diet manufacture carbon footprint was reduced by 14%. In a fully sustainable manner, CFP takes the protein co-product from a dry grind bioethanol plant to a new value proposition, expands market opportunities for bioethanol co-products, is already in production with resilience and redundancy in the supply chain and provides a valuable alternative concentrated protein for the livestock industry.

Key Words: protein, poultry, bio-ethanol, yeast, sustainability

Metabolism and Nutrition: Enzymes, Feed Additives

M86 Examining the effects of dietary calcium level and a multi-carbohydase on the performance of male broilers Reed Dillard¹GS, Adam Davis¹, Elizabeth Freeman¹, Ariel Bergeron², Robert Shirley² ¹University of Georgia, Poultry Science Department, ²Adisseo USA, Inc.

This 49d study evaluated the performance of male broilers that were fed diets consisting of corn-SBM-DDGS-wheat midds. Diets were formulated to contain three dietary Ca levels (low, medium, or high), resulting in three treatment feeds for each of the following: Positive Control (PC), Negative Control (NC), and NC supplemented with a multi-carbohydase enzyme (Rovabio® Advance TFlex; NCRA). The PC diets were formulated to the same AME and digestible AA level. The NC feeds were reformulated to ~2.43% less AME and ~2.64% less dAA. Dietary Ca was set at 0.80, 0.875 or 0.95% Ca in the starter phase (0-14d); the level of Ca was reduced by 0.05% for each of the three Ca levels as the phases changed to grower (15-28d), finisher (29-42d), and withdrawal (43-49d). The ratio of Ca to nPP was maintained at 1.85 and a 6-phytase was supplemented at 1,000 FTU/kg feed throughout the trial. Increasing dietary Ca in the starter phase resulted in a quadratic FCR response (1.257 vs. 1.240 and 1.252), feeding the PC, NC and NCRA resulted in the respective separation in BWG (540, 526 and 526 g/bird) and FCR (1.225, 1.261 and 1.260 ($p < 0.05$)). From 0-28d, raising dietary Ca increased the respective FCR from 1.440 to 1.444 and 1.471 ($p < 0.05$). Comparing the PC, NC and NCRA, the degree of separation in BWG and FCR across the Ca levels remained ($p < 0.05$); however, the supplementation of RA numerically improved BWG and FCR vs. the NC birds. From 0-42d, raising the level of Ca increased the respective FCR (1.667 to 1.670 and 1.701). Comparing the PC, NC and NCRA, the respective FCR were 1.639, 1.721 and 1.674 ($p < 0.05$) and the BWG was higher and comparable between the PC and NCRA vs. the NC fed birds ($p < 0.05$). High variation in BWG and FCR, due to high mortality in the 43-49d period, resulted in numerically lower BWG and higher FCR as dietary Ca increased. Comparing the PC, NC and NCRA, BWG was higher and comparable between the PC and NCRA vs. the NC fed birds ($p < 0.05$); however, FCR remained the lowest for the PC (1.771), followed by the NCRA (1.814) and NC (1.874) fed birds ($p < 0.05$). These data indicate higher dietary Ca level negatively affects broiler performance and the use of a multi-carbohydase can restore performance when diets are reformulated to a lower level of AME and dAA.

Key Words: Calcium, calcium level, broilers, carbohydase, broiler performance

M87 Phytase and multicarbohydase interactions on broiler performance, processing yield, and nutrient digestibility from 1 to 42 days of age Joseph P. Gulizia¹GS, Jose I. Vargas¹, Susan M. Bonilla¹, Santiago J. Sasia¹, Sara Llamas-Moya², Tri Duong², Wilmer J. Pacheco¹ ¹Department of Poultry Science, Auburn University, ²Kerry Inc.

This study evaluated the interaction effects between phytase and a multi-carbohydase containing α -galactosidase activity (CAG) on broiler performance, processing yield, and nutrient digestibility from 1 to 42 d of age. A total of 2,250 d-old Ross 708 x YPM male broilers were distributed randomly in 90 floor pens and assigned to 9 treatments with 10 replicates per treatment. A positive control sufficient in phosphorus (PC-P) and a PC for energy (PC-E) with 100 kcal/kg more ME were formulated. A negative control (NC) was manufactured to contain 0.20% less available phosphorus and calcium than the PC-P and 100 kcal/kg less ME than the PC-E at each feeding phase. Six additional treatments contained 500 or 1,500 FTU/kg of phytase combined with 0, 0.1, or 0.2 g/kg of CAG in the NC diet. Feed intake and BW were determined at 14, 28, and 42 d of age, and FCR corrected for mortality. Ileal digesta was collected from 5 birds per pen on d 42 to analyze nutrient digestibility. On d 43, 10 birds per pen were processed to determine processing yields. Data were analyzed using the GLM procedure of JMP, and means separated using Tukey's HSD with statistical significance considered at $P \leq 0.05$. Broilers fed the PC-E had improved BW and FCR compared to broilers fed the NC during the entire experiment ($P < 0.05$). Combining 0.2 g/kg of CAG with either 500 or 1,500 FTU/kg of phytase resulted in similar FCR to broilers fed the PC-E, and a 1.6- and 3.0-point improvement when compared to the non-CAG supplemented groups, respectively. Broilers fed a diet with 1,500 FTU/kg of phytase and 0.2 g/kg of CAG had 3.4 and 2.7% higher fillet yields compared to the NC and PC-E, respectively ($P < 0.001$). Crude protein digestibility (82.53 vs 77.21%; $P = 0.002$) and apparent ileal digestible energy (3,585 vs 3,254 kcal/kg; $P < 0.001$) improved when broilers were fed both enzymes at their highest inclusion rate compared to the NC. Regardless of CAG supplementation, phosphorus digestibility improved an average of 46.7 and 59.1% when broilers were fed a diet with 500 or 1,500 FTU/kg of phytase, respectively, compared to the NC ($P < 0.001$). Overall, supplementation of phytase and CAG improved broiler performance, processing yields, and nutrient digestibility in energy and phosphorus deficient diets.

Key Words: phytase, α -galactosidase, broilers, energy, phosphorus

M89 Effects of a novel consensus bacterial 6-phytase variant on growth performance of broilers fed corn-soybean meal based diets with meat and bone meal and deficient in nutrients and energy

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This study evaluated the effects of a novel consensus bacterial 6-phytase variant (PhyG) on growth performance of broilers fed US type conventional diets with reduced nutrients and energy. Four treatments were tested with 10 replicate groups, each containing 270, day-of-hatch Ross 308 male broilers. The diets were: 1) a nutrient-adequate control (PC) and 2), a nutrient-reduced negative control (NC): PC minus 0.19% dig P, 0.23% Ca, 0.045% Na, up to 0.05% unit dig AA and 37 kcal/kg ME and 3) NC with PhyG supplemented at 1,500 FTU/kg (PhyG1500) 4), PC minus 0.164% dig P, 0.201% Ca, 0.037% Na, up to 0.035%-unit dig. AA and 29 kcal/kg ME plus the PhyG at 750 FTU/kg (PhyG750). Xylanase was included in all diets at 2000 XU/kg with 25 kcal/kg of ME matrix applied. Diets were pelleted and based on corn-soybean meal with DDGs and meat and bone meal. Body weight (BW), body weight gain (BWG), average daily gain (ADG), and average daily feed intake (ADFI) of birds from 1 to 42 days were measured for each pen per phase and mortality corrected FCR was calculated. Data were analyzed using the fit model platform of JMP 15.1. Tukey's HSD was used for means separation. Compared with PC (1.60), the overall d 1-42 FCR was increased ($P<0.05$) by NC (1.71) and was maintained by PhyG750 (1.58) and PhyG1500 (1.58). The d 42 BW was

decreased ($P<0.05$) by NC (2,769 g) compared with PC (3,026 g) and the PhyG750 (3,020 g) and PhyG1500 (3,037 g) alleviated the adverse effect of NC. Similarly, PhyG750 (70.8 g/bird/day) and PhyG1500 (71.2 g/bird/day) treatments increased ($P<0.05$) ADG vs. NC (64.8 g/bird/day) to the same levels as PC (71.0 g/bird/day). PhyG treatment at each dose-level produced growth performance measures that were by phase and cumulatively equivalent to PC. For all measures, response values were numerically highest with 1500 FTU/kg. The NC+PhyG750 and the NC+PhyG1500 reduced the feed cost/kg BWG by 3.2% and 4.3% vs. PC, respectively. In conclusion, the novel consensus phytase variant completely compensated for the reduction in dig P, Ca, Na, dig AA, and ME respectively at each dose level and maintained growth performance of broilers fed conventional diets through 42 days of age, leading to reduced feed cost and production benefit.

Key Words: phytase, broiler, growth performance, full matrix

M91 Nutrient utilization, oocyst shedding and intestinal lesion in Eimeria-challenged broiler chickens fed diets with different levels of xylo-oligosaccharides Yang Lin^{*GS}, Oluyinka Olukosi
 University of Georgia

A total of 252 Cobb 500 male broiler chicks were used in a 21-d experiment to study the possibility of xylo-oligosaccharides (XOS) helping to recover gut impairment and depressed nutrient utilization in *Eimeria*-challenged broilers by improving nutrient utilization, gut permeability and intestinal lesion. Birds were allocated to 6 treatments in a 3×2 factorial arrangement (3 corn-soybean meal diets with 0, 0.5, or 1.0 g/kg XOS;

with or without *Eimeria* challenge). Each treatment had 6 replicates with 7 birds per replicate. Challenged groups were inoculated with a solution containing *E. maxima*, *E. tenella*, and *E. acervulina* oocysts on d 15. On d 21, ileal digesta and excreta were collected for nutrient utilization measurement and oocyst shedding evaluation. A 0 to 4 (no lesion to severe lesion) scale grading was used to score the coccidia lesion severity in upper (duodenum), middle (jejunum and ileum), and ceca sections of the digestive tract. *Eimeria* challenge resulted in severer ($P < 0.01$) intestinal lesion and greater ($P < 0.05$) oocyst shedding whereas XOS supplementation had no effect. There was no significant *Eimeria* \times XOS interaction for nutrient utilization responses. XOS supplementation at 0.5 g/kg tended to alleviate *Eimeria*-induced depression in ileal DM ($P = 0.052$) and N ($P = 0.104$) digestibility. Birds challenged by *Eimeria* had lower ($P < 0.01$) AME and total retention of DM, N, Ca, and P. On the other hand, supplemental XOS at 0.5 g/kg increased ($P < 0.05$) the total tract retention of DM, N, energy, and P; and tended to increase AME ($P = 0.051$) and Ca retention ($P = 0.062$). 1 g/kg XOS had no effect on nutrient utilization. In conclusion, 0.5 g/kg XOS supplementation helped recover depression in nutrient utilization from *Eimeria* challenge. Because of the lack of XOS effect on *Eimeria*-induced gut damage and oocyst shedding, it was hypothesized that part of XOS-associated performance recovery might be due to enhancement of digestible nutrient intake and this aspect with respect to carbohydrases and XOS effects are being investigated.

Key Words: xylo-oligosaccharides, *Eimeria*, nutrient utilization, broiler chicken

M93 Longitudinal characterization of the effect of probiotic, phytotherapeutic, and antimicrobial feeding strategies on poultry performance Ana Fonseca^{*GS}, Stephanie Clouser, John Boney, Erika Ganda *The Pennsylvania State University*

For decades, poultry producers have used antimicrobial supplementation in broiler diets to improve feed efficiency, growth rate, and intestinal health. However, consumers' apprehension and governmental regulations regarding antimicrobial resistance have led the poultry industry towards increasingly antimicrobial-free systems. Therefore, natural additives such as probiotics and phytotherapeutics are promising replacers to antimicrobial growth promoters. The purpose of this project was to longitudinally characterize the effect of a probiotic (*Bacillus subtilis*), a phytotherapeutic (organic oil blend), an antibiotic (bacitracin methylene disalicylate), and a basal diet on broilers performance. Three hundred and twenty Cobb 500-day-old chicks were randomly allocated in 32 cages with eight replicates of ten broilers per cage. Animals remained in the study from the day of hatch through day 21. The treatments consisted of four different experimental diets: a basal diet (control), treatment 1 (probiotic), treatment 2 (phytotherapeutic), and treatment 3 (antibiotic). Bodyweight (BW), Average daily gain (ADG), Feed intake (FI) and Feed conversion ratio (FCR) were recorded on days 10 and 21. Probiotic administration improved FCR between days 0-10 (-0.12 (-8.5%), $P = 0.05$) and 0-21 (-0.05 (-3.5%), $P < 0.05$) compared to the control diet. Numerically, all additives obtained better results on BW, ADG, and FI compared to the control diet. No significant differences were observed in any outcomes between probiotic, phytotherapeutic, and antibiotic groups. Overall, probiotic and phytotherapeutic additives seem positively to affect broilers' growth performance. Therefore, having a closer understanding of the role of these products on broilers' microbiota, will be an important key to creating direct strategies to improve broilers' performance and gut health.

Key Words: broilers, feed, additives, performance

M94 Efficacy of multienzyme supplementation on growth performance, nutrients digestibility, gut health and microbiome of broiler chickens infected with *Eimeria* species. Jing Yuan^{*GS}, Timothy Johnson, Kolapo Ajuwon, Olayiwola Adeola *Department of Animal Sciences, Purdue University*

Avian coccidiosis is an infectious parasitic disease that triggers severe intestinal inflammation, leading to nutrient malabsorption, bleeding, and even death. Here we evaluated the response of coccidia-challenged birds to dietary multienzyme (phytase, xylanase, β -glucanase, amylase, hemi-cellulases, and pectinases) supplementation. In a 21-day trial, Ross 308 broiler chickens were allotted to a 2 \times 2 factorial arrangement consisting of 0 or 25 g/kg multienzyme and challenge with PBS or *Eimeria* oocysts. Coccidia challenge by oral gavage with multiple *Eimeria* spp. oocysts (50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina*) was introduced on day 13 post hatching. The multienzyme alleviated ($P < 0.05$) *Eimeria*-induced reduction in feed efficiency and nutrient utilization. Multienzyme supplementation reversed ($P < 0.05$) the upregulation of pro-inflammatory cytokine IL-8 in duodenum and jejunum resulting from *Eimeria* spp. infection. The expression of amino acids transporter b⁰,+AT was upregulated ($P < 0.05$) in the jejunum following multienzyme treatment. The ileal expression of antioxidant HMOX1 was induced ($P = 0.05$) in the multienzyme group with coccidia challenge. Coccidiosis decreased ($P < 0.05$) the diversity and modulated the composition of microbial communities in ileum and ceca. Coccidia challenge facilitated ($P < 0.01$) the colonization of opportunistic pathogens such as *Actinobacillus*, *Bacillus*, and *Streptococcus*, and inhibited ($P < 0.01$) the growth of cecal beneficial microbiota such as *Bifidobacterium*, *Clostridia*_UCG-014, and *Facecalibacterium*. Multienzyme supplementation increased ($P < 0.01$) the proliferation of beneficial bacteria, especially SCFA-producers and fiber-degrading genus, such as *Anaerostipes*, *Butyricicoccus*, and *Pseudoscardovia*. In conclusion, multienzyme supplementation partially reversed the detrimental effects of coccidiosis by improving nutrient utilization,

exerting anti-inflammatory effects, upregulating the expression of amino acid transporter, and restoring microbial homeostasis, and could be a potential strategy to control coccidiosis in poultry.

Key Words: broilers, coccidia challenge, multienzyme, gut health, microbiota

M95 Effects of non-starch polysaccharide degrading enzyme inclusion to corn and soybean-meal based diets containing wheat on broiler performance and ileal energy digestibility Courtney Poholsky^{*1GS}, Alyssa Lyons¹, Staci Cantley², John Boney¹ ¹*Pennsylvania State University*, ²*Huvepharma Inc.*

Wheat may be included in corn and soybean-meal based (corn/soy) diets when it is available and cost effective. The non-starch polysaccharide (NSP) content in wheat must be considered. Enzyme inclusion can enhance nutrient utilization and broiler performance when properly selected based on dietary substrates. A 39d study was conducted to determine the ileal digestible energy (IDE) and performance effects of two NSP degrading enzymes when added to corn/soy diets containing 20% wheat. A total of 1,152 Ross 708 straight-run broilers were housed in 12 replicate floor pens per treatment. Dietary treatments included: breed specification (PC), 100 kcal/kg energy reduction (NC), NC + Product A (single-strain, xylanase enzyme with residual side-chain activity), and NC + Product B (single strain enzyme with defined xylanase, xylo-glucanase, and β -glucanase activities). Treatments were arranged in a randomized complete block design with a pen of 24 broilers serving as the experimental unit. For each diet phase, a master batch was made and split into four allotments: one allotment for the PC diet and the other three allotments for the NC diets. Either sand, Product A, or Product B were added to NC diets at 0.01%. Titanium dioxide (TiO₂) was added at 0.020% to all diets in the Finisher 1 period to determine IDE. On d37, distal ileal contents from three birds per pen were collected. Feed and digesta samples were analyzed for TiO₂ and energy content. The GLM procedure of SAS was used to conduct one-way ANOVA. Day 1-24 performance results showed that birds fed the PC diet had improved FCR compared to all other treatments ($P < 0.01$). From d1-39, birds fed the NC + Product B diet improved FCR compared to birds fed the NC diet ($P < 0.01$); however, Product B supplementation did not improve FCR to that of the PC fed birds ($P > 0.05$). Birds fed the NC + Product B diet improved IDE by 119 and 115 kcal/kg above birds fed the NC diet and NC + Product A supplemented diet, respectively ($P < 0.01$). These data indicate that addition of Product B to NC diets containing 20% wheat was more effective at improving IDE and FCR compared to the addition of Product A. Proper enzyme selection, based on substrate availability when wheat is included to corn/soy diets, may offer performance and IDE benefits.

Key Words: wheat, enzyme, energy, digestibility, broiler

M96 The effect of cellulose fiber, wheat bran, and cotton carpal fiber at different grind sizes on the cecal microbiome of both uninfected turkey poults and turkey poults challenged with *Clostridium perfringens* Olivia Wedegaertner^{*GS}, Aaron Kiess, Robert Beckstead, Chongxiao Chen *North Carolina State University*

The avian ceca are a pair of blind pouches that arise from the junction of the ileum and colon. Small undigested feed particles, digestive fluid and urine are refluxed via reverse peristalsis from the colon into the ceca for microbial fermentation. The typical poultry diet contains little fiber because it does not directly deliver any nutritional value to the bird but is naturally directed into the cecal lumen and may be used to manipulate the cecal microbiome. Two trials were conducted to evaluate the effect of various dietary fiber on the cecal microbiome. In trial 1, 720 day-old turkey poults were divided into 15 dietary treatment groups (12 birds/pen, 4 reps/trt): cellulose fiber, wheat bran, CCF (cotton carpal fiber) at 3 grind sizes, CCF + oil, and oil alone, each at a 1% or 5% inclusion level, and a control diet. Cecal content was collected at 4 weeks for 16s microbiome

analysis. Data were analyzed using one-way ANOVA (JMP 14, $P < 0.05$). Differences among treatments were observed at the genus and species level in both inclusion levels. Specifically, at 1% lower *C. perfringens* counts ($p = 0.0046$) associated with the control, cellulose, CCF coarse grind, CCF + oil, and CCF superfine grind treatments were found compared to the wheat bran treatment. *Lactobacillus* was highest in concentration when fed the 5% CCF superfine grind dietary treatment ($p = 0.0423$) compared to the control, cellulose, and oil treatments. In trial 2, 40 two-day-old turkey poults were placed into 2 dietary treatment groups: control and treatment feed with CCF superfine grind at 1% (20 birds/trt) and challenged with *C. perfringens* on D7. Cecal contents was collected for microbiome analysis. *Lactobacillus* counts were significantly higher ($p = 0.0291$) in birds fed the dietary treatment than the control diet. Although nonsignificant ($p = 0.6836$), the treatment diet (358.00 OTU) did result in reduced *C. perfringens* counts in the ceca of challenged birds than the control diet (548.00 OTU). Trials 1 and 2 suggest that certain dietary fibers may facilitate elevated counts of *Lactobacillus* in the cecal lumen due to the increase in fermentable material while also reducing *C. perfringens*, supporting the possible use of CCF fibers as both a dietary fermentation stimulant and a natural antibacterial agent.

Key Words: dietary fiber, cecal fermentation, cecal microbiome, phytonutrients, *Clostridium perfringens*

M97 Plant extracts and tributyrin response on young laying hen performance Jordyn Samper^{*1GS}, Brian Glover², Michael Persia¹, Jose Charal², Milan Hruby² ¹*Virginia Tech*, ²*Archer Daniels Midland Company*

An experiment was conducted to determine the effects of a commercial plant extract preparations based on capsicum, carvacrol, and cinnamaldehyde (C3; Xtract® 6930) and esterified butyric acid – tributyrin (EBA; Daaforce® 100) on layer performance during pre- and peak production period. Past research has shown nutrient digestibility improvement with C3, and overall poultry performance impacted by C3 and EBA, with limited young layer data. 144 Hy-Line W-36 laying hens were kept in cages in the study conducted between 23 – 43 weeks of age. Birds were assigned to 4 treatments: 1) control, 2) control plus C3 (0.1 g/kg), 3) control plus EBA (0.5 g/kg), and 4) control plus a combination of C3 and EBA, each at ½ dose of inclusion used for each product in treatments 2 and 3. There were 12 replicate cages per treatment with 3 birds per cage. Birds were offered a mash diet based on corn, soybean meal, poultry by-product meal and DDGS. The feed intake was controlled at 95 g per day. Mortality, egg production and egg mass were recorded daily. All eggs were used for average egg weight measurement. Feed intake and feed efficiency were recorded every two weeks. Eggs were collected for five consecutive days every four weeks for egg analyses. Egg component weights, yolk color, Haugh unit, shell breaking strength and specific gravity were evaluated every 4 weeks on 3 eggs collected per treatment. The study was performed during April through August 2020. Data were analyzed using Statistix 10.0 software (Analytical Software, Tallahassee, FL) with mean separated by LSD test. C3 or EBA at full dose significantly ($P < 0.05$) improved egg production (94.31, 94.14 vs. 91.6%), feed efficiency (585, 594 vs. 568 g egg mass/kg feed), egg mass (54.68, 55.04 vs. 52.35 g/d) and egg weight (58.01, 58.46 vs. 57.26g) versus control treatment. A combination of C3 and EBA based on a half dose of each product, significantly ($P < 0.05$) improved egg mass and egg production compared to control treatment and numerically improved feed intake, egg weight and feed efficiency. Shell weight was significantly ($P < 0.05$) increased with C3 treatment. The results of this study suggest the opportunity for C3 and EBA to be used successfully in layers during pre- and peak period to contribute to optimal performance.

Key Words: layer, plant extracts, butyric acid, combination, performance

Metabolism and Nutrition: Amino Acids

M99 Evaluating the impact of varying digestible lysine and AME levels fed during the finisher phase (28-41 d) on male Cobb MV × Cobb 500 FF broiler chickens growth performance and processing yield Dalton Dennehy^{*1GS}, Andrew Brown¹, Cesar Coto², Leonel Mejia², Kelley Wamsley¹ ¹*Department of Poultry Science, Mississippi State University, Cobb-Vantress*

Previous research using male Cobb MV x Cobb 500 FF broilers has shown that varying starter and grower digestible lysine (dLys) and energy (AME) levels can impact performance and processing. Therefore, the objective of this study was to evaluate the effect of feeding varying dLys and AME levels during the finisher phase (d 28-41). A 3 dLys (Low-0.87, Med-0.97, and High-1.07%) × 4 AME (Low-3030, Med-3120, High-3210, and Very High-3300 kcal/kg) factorial arrangement was utilized. Chicks were sourced from a commercial hatchery, vent sexed, and randomly assigned to 96 pens (0.086 m²/bird). Birds were fed common starter and grower (1 and 2) diets (1.22% + 2975, 1.12% + 3025, and 1.02% dLys + 3100 kcal/kg, respectively) until d 28. Measured variables included BWG, FCR, ADFI, average daily total lysine and gross energy intake (ALI and AGEI, respectively), and d 41 processing. Feeding Med or High dLys maximized d 28-40 BWG (P=0.004) regardless of AME level fed. As dLys increased, a stepwise reduction in FCR and ADFI, as well as an increase in ALI was observed from d 28-40 (P<0.05). For the main effect of AME, broilers fed Very High AME improved FCR at d 28-40 (P<0.0001), with those fed Low AME having a higher FCR and birds fed Med and High being intermediate. Birds fed Low AME had the highest ADFI throughout but were similar to Med at d 28-35 and 28-40, as well as Med and Very High at d 35-40 (P<0.05). Also, birds fed Very High AME had the highest ALI but were similar to those fed Med at d 35-40 and 28-40 (P<0.05). An interaction was detected for carcass yield (P=0.013) in which broilers fed Low dLys + Low AME had the lowest yield but similar to those fed Low dLys at all other AME levels, Med dLys + High and Very High AME, and High dLys + Very High AME. Feeding High dLys maximized breast and tender yield and reduced fat pad yield (P<0.05). For the main effect of AME, fat pad yield was greatest in birds fed High and Very High AME (P=0.016). Overall, increasing d 28-41 dLys improved performance and processing variables; Very High AME level improved FCR and ADFI. These data demonstrate the importance of evaluating a particular broiler strain's response to varying dLys and AME levels, as these nutrients can impact performance and ultimately producer profitability.

Key Words: broiler, digestible lysine, energy, performance, processing

M100 Can soybean meal be replaced with canola meal and corn DDGS in low crude protein diets supplemented with crystalline amino acid? Adeleye Ajao^{*GS}, Dima White, Woo Kim, Oluyinka Olukosi *University of Georgia*

A 42-day study was done to explore the application of supplemental amino acids (AA) in reduced crude protein (CP) diets with SBM, CM or DDGS as the main protein feedstuffs. Responses of interest were growth performance, carcass yield, whole-body composition, litter ammonia and litter N. A total of 810 Cobb 500 male broilers were randomly allocated to 54 floor pens, with 15 birds per pen on d 0. All the birds received the same starter diets (22% CP, 3000 kcal/kg AME) during the first 10d. Thereafter, 9 experimental diets (D) were provided in both grower (d10-28, 18.5% vs 14% CP for adequate and low CP, respectively) and finisher (d28-42, 17% vs 13% CP for adequate and low CP, respectively) phases -; D1 was adequate CP corn-SBM positive control diet. The CP of D2 and D3 (corn-SBM based) was decreased by 2 or 4.5%, respectively relative to D1. Diets-, D4, D5 and D6 had the same CP levels as D3 but with increasing DDGS inclusion levels (5%, 8.75%, 12.5%, respectively). Diets D7, D8 and D9 had the same CP level as D3 but with CM inclusion levels of 5%, 7.5%, 10% respectively. Diets 2 to 9 (the low-CP diets) had same level of standardized ileal digestible indispensable AA with supplemental indispensable AA added as required. Gly and Ser were added as sources of N. Bird weight and feed intake were measured on d 10, 28 and 42. Whole body composition was determined using dual energy x-ray absorptiometry on d 21 and 42, whereas litter N, ammonia and carcass yield were determined on d 42. Serial reduction in dietary CP with SBM linearly reduced (P < 0.05) weight gain, feed intake and gain to feed. Increasing levels of DDGS and CM, at constant CP level, also linearly (P < 0.05) reduced weight gain, feed intake and gain to feed ratio. Breast yield linearly reduced (P < 0.05) with serial reduction in CP with SBM, but no significant differences were observed between SBM, DDGS or CM diets at the same CP and AA levels. Litter N and ammonia increased (P < 0.05) with increasing levels of CP in SBM diets. In conclusion, 5% inclusion levels of CM at the same low-CP levels as SBM produced similar growth response, whereas higher inclusion levels of CM and DDGS were detrimental; hence complete replacement of SBM with DDGS or CM in low-CP diets was not feasible for optimum performance.

Key Words: Low crude protein, Amino acid, Canola, Soybean, corn DDGS

M101 Reducing dietary crude protein through inclusion of valine, arginine, and isoleucine maintains broiler performance, yield and reduces nitrogen excretion Mitchell Davis^{*1GS}, Roshan Adhikari², Jason Lee², Adam Davis¹ ¹The University of Georgia, ²CJ America Inc.

With the recent commercialization of feed grade crystalline valine, (V), arginine (R), and isoleucine (I), nutritionists can use them to reduce dietary crude protein (CP) and diet cost. But there is limited data evaluating growth performance and processing yields when achieving elevated CP reduction using 6 crystalline amino acids (AA) in the diet. The objective of this study was to evaluate if use of commercially available V, R and I would maintain growth performance and processing yield while achieving reduced CP and cost savings. For this, 2,160 day of hatch, Ross 708 broilers were assigned to 5 treatments and distributed randomly to 80 floor pens. Treatment 1 (T1) consisted of a standard corn/soybean meal/distillers dried grains with solubles-based diet with added crystalline forms of lysine (K), methionine and threonine meeting or exceeding all essential AA requirements. Treatments 2-4 were similar in composition to T1 except crystalline V, R, and I were sequentially added to each subsequent dietary treatment to reduce CP and meet requirements of the 4th, 5th and 6th limiting AA, respectively. Treatment 5 was similar to T4 except crystalline glycine (G) was added to uplift the dG+S:dK similar to T1. Broilers were fed starter, grower, and finisher diets for consecutive 2-wk intervals, and fed a withdrawal diet from 42 to 49 d of age. Body weight and feed consumption were determined at the end of each dietary phase. On d 49, 7 broilers per pen were selected and processed, foot pads were visually scored for lesions on all birds and the nitrogen content of the litter was determined. For the overall 49-d experimental period, body weight, body weight gain, and feed to gain, as well as a total white meat, wing or leg quarter yield did not differ between the broilers in T1, T2, T3 and T4. Treatment 5 had a feed to gain value that was greater ($P > 0.05$) than the 4 other treatments, but foot pad score and litter moisture were lower for T5 compared to the other 4 treatments. The results indicate that broiler diets can be formulated using V, A and I to reduce CP and balance the 4th, 5th and 6th limiting AA requirement without any loss in live performance or processing yields, and at the time of the experiment, the dietary cost of T2-3 was less than T1 in all phases.

Key Words: footpad health, litter quality, feed cost, performance, amino acids

M102 Responses of broilers to increasing dietary glycine when fed reduced or low crude protein diets from 0 to 48 days Trevor Lee^{*1GS}, Jason Lee², Chuanmin Ruan¹, Samuel Rochell¹ ¹Center of Excellence for Poultry Science, University of Arkansas System Division of Agriculture, ²CJ America, INC

Two experiments evaluated broiler performance and processing characteristics when fed increasing Gly concentrations in reduced (RCP) or low CP (LCP) diets from 0-48 d. Ross 708 male off-sex chicks reared in floor pens (21 birds/pen) were used in experiment 1 (EXP 1; 1,008 chicks) and 2 (EXP 2; 1,260 chicks). EXP 1 included 4 treatments: a control (CTL) diet and a RCP diet (~1.7% unit CP reduction) without or with Gly added to achieve 100 (moderate; M Gly) or 112% (high; H Gly) total Gly+Ser of the CTL diet in each phase. EXP 2 included 6 treatments: a CTL diet, a RCP diet, or a LCP diet (~2.4% unit CP reduction and 88% total Gly+Ser; LCP88) without or with Gly added to achieve 100, 112, or 124% total Gly+Ser of the RCP diet. Both EXP included starter (0-14 d), grower (14-28 d), and finisher 1 (28-39 d) and 2 (39-48 d) diets with similar digestible essential amino acid levels. Treatments were replicated in 12 pens in EXP 1 and 12 or 9 pens in EXP 2. Dietary nutrients met breeder specifications. Body weight gain (BWG), feed intake (FI), and FCR were evaluated, and 6 birds/pen were randomly selected for processing at 48 d. Data from EXP 1 were analyzed by a 1-way ANOVA and Tukey's means separation, while data from EXP 2 were analyzed by pre-planned orthogonal, linear, and quadratic contrasts ($P \leq 0.05$). In EXP 1, 0-14 d broiler performance was similar ($P > 0.05$) among treatments. From 0-48 d, broilers fed the H Gly

diet had the lowest ($P=0.006$) BWG and highest ($P=0.003$) FCR. Feeding either the RCP or M Gly diet resulted in growth and processing yields similar ($P > 0.05$) to the CTL. In EXP 2, increasing Gly in the LCP88 diet linearly reduced ($P \leq 0.027$) 0-14 d FI and FCR with no effect on BWG. From 0-48 d, broilers had similar ($P > 0.05$) performance when fed the CTL or RCP diets, but had a higher ($P < 0.001$) FCR when fed the LCP88 diet. Increasing Gly linearly reduced ($P=0.033$) FCR. Total breast meat yield was reduced ($P \leq 0.02$) for birds fed the LCP88 diet relative to that of CTL and RCP-fed birds and did not respond to increasing Gly. Cumulative data from these 2 EXP indicate that Gly needs of broilers reared to market ages may depend upon dietary CP content *per se* or other dietary changes associated with low CP diets.

Key Words: amino acid, glycine, serine, reduced crude protein, broiler

M103 Reduced crude protein diets maintains growth and yield parameters of mixed-sex broilers while reducing nitrogen excretion, litter moisture, and ecological footprint Oscar Machado¹, Leonel Mejia², Victor Naranjo^{*3}, Wilmer Pacheco⁴, Yordan Martinez¹ ¹Zamorano University, ²Cobb-Vantress, ³Evonik Guatemala S.A., ⁴Auburn University

A study was conducted to determine the effects of crude protein (CP) reduction with amino acid (AA) balanced diets on growth, carcass yields, litter characteristics, and ecological footprint of feeds in mixed-sex broilers. A total of 2,688 Cobb MV × 500 FF broilers were allotted to 4 treatments (14 replicates of 48 birds per pen) from 11 to 36 d of age reared in an open-sided barn. Dietary treatments included: 1) Control diet (C) with DL-Met+L-Lys+L-Thr, 2) C+L-Val 3) C+L-Val+L-Ile and 4) C+L-Val+L-Ile+L-Arg. Treatments in mash form were offered in the grower (11 to 25 d) and finisher (26 to 36 d) phases. Crude protein in the grower phase (SID Lys = 1.12%; AMEn = 3,100 kcal/kg) were 21.7, 20.5, 19.4 and 18.2% while in the finisher (SID Lys = 1.02%, AMEn = 3,180 kcal/kg) were 19.6, 19.3, 17.9 and 17.4%, respectively. Nitrogen (N) utilization was calculated using feed intake, CP of diets, final BW and assuming 30 g N/kg BW. Composite litter samples were collected from 4 replicate pens per treatment at 25 and 35 d of age and analyzed for pH, water, N-NH₃, and K⁺ contents. The ecological footprint of feeds was calculated using GABI database. Data were analyzed using one-way ANOVA and means were separated by Tukey's HSD with statistical significance considered at $P \leq 0.05$. Overall (11 to 36 d) reduction of CP did not affect ($P > 0.05$) BW gain, feed intake or mortality. Feed conversion was similar across treatments up to C+L-Val+L-Ile, but C+L-Val+L-Ile+L-Arg had increased ($P \leq 0.05$) FCR compared to the control. Final BW and carcass parameters as percentage of BW were not affected ($P > 0.05$). Overall N-utilization was improved (66 vs. 59%) and N excretion reduced (28 vs. 39 g/bird) in C+L+Val+Ile+L-Arg compared to the control. Reduction of dietary CP reduced litter's moisture ($P = 0.02$), K⁺ ($P = 0.02$) and tended to reduce N-NH₃ ($P = 0.06$). Relative to control, the climate change with land use (kg CO₂ eq.) footprint of C+L+Val+Ile+L-Arg was reduced by 18 and 12% in the grower and finisher phase, while the eutrophication (kg P eq.) potential was reduced by 15 and 10%. Reduction of CP with an appropriate use of supplemental AA maintained growth and carcass yield parameters while improving litter quality, N-utilization and the sustainability of broiler production.

Key Words: amino acids, broiler, crude protein, nitrogen, sustainability

M104 Interactive effects of dietary isoleucine and valine ratios to lysine in response to varying leucine to lysine ratios in broilers Ruben Kriseldi^{*1}, Alejandro Corzo¹, Marcelo Silva¹, Jason Lee², Roshan Adhikari², Chance Williams³ ¹Aviagen, ²C.J. Bio America, ³Wayne Farms

A central composite design (CCD) study was conducted to understand the relationship among dietary branched-chain amino acids (BCAA) on live performance, carcass traits, and feather composition in Ross 344 × 708 male broilers. A total of 2,592 d-old male chicks were randomly placed

into 144-floor pens. Each pen received 1 of 16 dietary treatments from 20 to 35 d of age varying in digestible ratios of Ile:Lys (52 to 75), Val:Lys (64 to 87), and Leu:Lys (110 to 185) arranged as CCD. Birds and feed were weighed at 20 and 34 d of age to determine body weight gain (BWG), feed intake, and feed conversion ratio (FCR). At 35 d of age, all birds except 1 were processed to determine carcass characteristics. Feather dry matter, protein, and amino acid compositions were determined from the last 5 primary feathers of 1 bird per pen. Data were analyzed as CCD using the surface response option of JMP v.15. Body weight gain (1,332 g; $P < 0.001$; $R^2 = 0.93$) and FCR (1.54; $P = 0.002$; $R^2 = 0.88$) from 20 to 34 d of age were optimized at the lowest Leu:Lys ratio (110) with minimum Val:Lys (78 to 79) and Ile:Lys (65 to 66) ratios. Poorer BWG and FCR were observed as Leu:Lys ratio increases although increasing Val:Lys and Ile:Lys ratios alleviated the poor performance. Opposite to live performance, both carcass (71.5%; $P = 0.031$; $R^2 = 0.76$) and breast yield (26.7%; $P < 0.001$; $R^2 = 0.96$) were maximized at the highest Leu:Lys ratio. This effect was complemented by increasing Ile:Lys ratio beyond 68. In contrast, it requires lower Ile:Lys and Val:Lys ratios to maximize carcass and breast yield at the lowest Leu:Lys ratio but with lower meat yields than in high Leu:Lys ratio. Feather protein content was the highest (96.1%) when increasing Ile:Lys ratio to 70 ($P = 0.07$) with Leu:Lys and Val:Lys ratios of 110 and 80, respectively. Increasing Leu:Lys ratio to 185 decreased maximum feather protein content to 93.5%. These results revealed that optimum BCAA ratios to Lys may vary depending on the desired response criteria and demonstrate the importance of maintaining proper Val and Ile ratios dependent on dietary Leu. Live performance can be optimized in diets with low Leu:Lys ratios; however, meat yield can be enhanced by increasing dietary Leu:Lys along with Ile:Lys ratios.

Key Words: leucine, isoleucine, valine, central composite design, broiler

M105 Standardization of the KOH protein solubility test for soybean meal in a single commercial laboratory and correlation to *in vivo* poultry amino acid digestibility Nelson Ruiz¹, Carl Parsons², Brian Steinlicht³, Dave Taysom³, Xin Wu⁴, Fiona Liu⁴ ¹Nelson Ruiz Nutrition, LLC, ²Department of Animal Sciences, University of Illinois, ³Dairyland Laboratories, Inc., ⁴American Oil Chemists' Society

The KOH protein solubility (KOHPS) test is used to evaluate whether a lot of commercial soybean meal (CSBM) has undergone the Maillard reaction during processing. The method was introduced to the animal feed industry in 1990 by Araba and Dale and is widely used worldwide. However, there are at least two limitations to this important measure of CSBM quality that are still poorly explained: First, large inter-lab variability is routinely observed. Second, nutritionists and formulators are unable to estimate the amino acid digestibility (AAD) for lysine (LYS) and arginine (ARG) in lots of CSBM affected by the Maillard reaction because a quantitative interpretation of the results in terms of AAD has not been developed. The objective of this study was to standardize the KOHPS test in a single commercial lab starting from the description by Araba and Dale (1990), utilizing CSBM samples for which the *in vivo* AAD was already known. Two CSBM samples, one with high solubility (HS) (KOHPS ~ 80-85%) and another with low solubility (LS) (KOHPS ~ 50-55%) were used as reference samples. After a preliminary protocol with the HS reference sample testing $\geq 80\%$ solubility was developed, adjustments in the intensity of agitation were made with 4 CSBM samples of known *in vivo* LYS digestibility (88-96% digestible LYS) to match their KOHPS values in the 80-85% solubility range. The resulting standardized protocol yielded 83.08% KOHPS for the HS CSBM reference sample (mean value of 72 test results) with repeatability standard deviation, 1.45%; repeatability relative standard deviation, 1.74%; (Within-Lab) reproducibility standard deviation, 3.91%; and (Within-Lab) reproducibility relative standard deviation, 4.70%. It also yielded 52.77% KOHPS for the LS CSBM reference sample (mean value of 71 test results) with repeatability standard deviation, 1.20%; repeatability relative standard deviation, 2.27%; (Within-Lab) reproducibility standard deviation, 5.32%; and (Within-Lab)

reproducibility relative standard deviation, 10.08%. In conclusion, a standardized protocol is available for the determination of KOHPS of CSBM that will allow the building of regression equations for the estimation of AAD to be used in least-cost formulation.

Key Words: KOH protein solubility, digestible lysine, digestible amino acids, soybean meal, Maillard reaction

M106 Precise nutrition: Regression equations using KOH protein solubility to estimate digestible lysine and digestible arginine in commercial lots of soybean meal that have undergone the Maillard reaction Nelson Ruiz¹, Carl Parsons², Benjamin Parsons² ¹Nelson Ruiz Nutrition, LLC, ²Department of Animal Sciences, University of Illinois

The objective of this study was to deliver a set of statistically significant regression equations (RE) for lysine (LYS) & arginine (ARG) that will allow poultry nutritionists & formulators to estimate the amino acid digestibility (AAD) of these amino acids (AAs) using a newly standardized protocol for the KOH protein solubility (KOHPS) test applied to commercial lots of soybean meal (CSBM). A total of 88 samples from a set of 100 previously analyzed for *in vivo* AAD and kept in frozen storage were analyzed for KOHPS. KOHPS values ranged from 86% to 13% while digestible LYS (DLYS) coefficients (COEFF) ranged from 0.96 to 0.47. Digestible ARG (DARG) COEFF ranged from 0.97 to 0.62. Because it became evident from the total LYS & total ARG analyses of the original samples that a net destruction of LYS & ARG occurred as KOHPS dropped. Therefore, previously published RE for total AAs regressed on protein content were used to calculate destruction-corrected LYS & ARG contents. Regressions of digestible COEFF on KOHPS were then run with & without corrected digestible COEFF for both AAs. Linear regression provided the best fit for KOHPS vs. corrected digestible COEFF for both LYS & ARG. One-slope broken line regression for LYS indicated that convergence criterion was met at KOHPS=80% & DLYS coefficient=0.88 meaning that an adequately processed CSBM lot by solvent extraction is optimum at a minimum of 80% KOHPS which in turn is correlated with a minimum *in vivo* DLYS of 0.88. Having a statistically significant RE between KOHPS & DLYS COEFF for CSBM contributes to the realization of precision nutrition. The data confirmed a long time observation that ARG is more digestible than LYS in CSBM, therefore by setting the minimum of 80% KOHPS for CSBM without Maillard reaction damage (overprocessing) on the basis of DLYS both AAs are at high AAD provided trypsin inhibitors are not in excess (underprocessing). In conclusion, the RE here reported are the following:

DLYS (coefficient*100) = $0.59 * \text{KOHPS} + 41.06$ (N=76; R-square=0.95; $r=0.97$; $P < 0.0001$)

DARG (coefficient*100) = $0.36 * \text{KOHPS} + 62.33$ (N=76; R-square=0.87; $r=0.93$; $P < 0.0001$)

NOTE: the utilization of these RE implies that the KOHPS values for CSBM have been generated using the newly standardized protocol.

Key Words: KOH protein solubility, digestible lysine, digestible arginine, soybean meal, Maillard reaction

M107 Interaction effects of amino acids relative to ideal lysine ratio and exogenous protease supplemented to low CP diet on performance of broiler chicken Muhammad Adeel Maqsood¹, Ehsaan Ullah Khan¹, Sohail Ahmad² ¹Department of Animal Nutrition, University of Veterinary and Animal Sciences, ²Department of Poultry Production, University of Veterinary and Animal Sciences

This study evaluated the effect of amino acids balanced at specific lysine ratio with exogenous protease supplementation along with a reduced CP levels in broiler diet. In total 480 straight run broiler chicks (Ross 308) were studied. A completely randomized design in factorial arrangement,

with 4 treatments of 6 replicates with 20 birds each, was applied. Treatments consisted of 2 lysine ratio (100% and 110%) without or with exogenous protease supplementation (200g/ton) along with a 20 % reduction of crude protein from standard requirements of Ross-308 (18.4% in starter and 17.2% in grower phase). Growth performance (feed intake, weight gain, feed conversion ratio, livability), carcass traits (live and carcass weight, carcass yield, heart, liver, gizzard, leg quarter, breast weight, and abdominal fat percentage), gut morphology (villus height, crypt depth, and villus height & crypt depth ratio), and serum metabolites (total protein, albumin, and uric acid) were evaluated. A significant interaction ($p \leq 0.05$) for lysine ratio and protease was noted in body weight gain, feed conversion ratio, carcass traits, and gut morphology throughout the experimental period, but did not influence ($p > 0.05$) serum metabolites. It was concluded that broiler diet with net reduction of 20% CP from Ross-308 standards, balanced for amino acids at lysine ratio 110% supplemented with exogenous protease could be used to improve growth performance, intestinal health, and carcass traits.

Key Words: Amino Acids, Broiler Performance, Exogenous Protease, Low CP Diet, Lysine ratio

M108 Reviewing nutritional standards for the modern male turkey: optimum digestible arginine to lysine ratio Roshan Adhikari¹, Jason Lee¹, Curtis Novak², Reza Poureslami², Jeff Firman³ ¹*CJ Bio America*, ²*Purina Animal Nutrition*, ³*Missouri Contract Poultry Research*

Depending on dietary ingredient profile, arginine can fall anywhere between the 4th and 6th in the order of amino acid limitation. However, an

arginine dose titration has not been published in many years, and with the continued genetic selection and growth performance improvements of commercial turkeys, it is necessary to reevaluate the optimum arginine ratio. Arginine is integral in various key metabolic pathways to enhance systemic immunity as well as local (intestinal mucosal) immunity of birds. The objective of this study was to determine the optimum dArg ratio of male turkey poults from 0-42d of age. A dose-response experiment was conducted using 1000 day-old toms in a completely randomized design allocated equally to 50 pens within 5 treatments. Corn, SBM, DDGS, and Corn gluten meal based pre-starter (0-21d), and starter (22-42d) diets were formulated to be deficient in Arg at 82% of dLys. L-Arg was gradually added to make 4 additional treatments at 92%, 102%, 112%, and 122% of digestible Arg to Lys ratio. Digestible Lys content, energy, and dTSAA were fixed in all treatments at 1.65%, 3086 Kcal/kg, and 1.16% in pre-starter diets and 1.54%, 3196 Kcal/kg, and 1.10% in starter diet respectively. All ingredients were analyzed for total amino acid content prior to formulation. Feed intake, body weight gain, feed conversion ratio was evaluated on d21 and d42. Minimum requirement of the dArg:dLys for optimum live performance was estimated using linear broken line (LBL) models. LBL regression suggests that the optimal dArg:dLys for FI and BWG through 42 days of age is 125%. Whereas, the optimal dArg:dLys for FCR through 42 days of age is 106 using LBL regression. The results of this study indicate that optimum Arg to Lys ratio to maximize growth performance in the young modern male turkey poult is higher than previously assumed.

Key Words: Turkey, Arginine, Requirement, Performance

Metabolism and Nutrition: General Nutrition

M109 Survey of US soybean meal - does region influence quality and antinutritional factors? Katie Hilton^{*}, Elizabeth Kim, Janet Remus, Franco Mussini *Danisco Animal Nutrition (IFF)*

Soybean meal (SBM) makes up approximately 40% of poultry diets. With recent increases in cost of this ingredient, evaluation of quality is critical. This study evaluated a total of 122 SBM samples collected from commercial feed mills, from nine different states, six different SBM suppliers, four different regions and included two separate harvest years (2019 and 2020). Samples were sent to two separate labs (University of Missouri agricultural experiment station chemical laboratories, and Eurofins) and analyzed for amino acids, digestibility factors, anti-nutritive factors and trypsin inhibitors. The model was a factorial in an 9 x 6 x 4 x 2 arrangement, consisting of nine states, six suppliers, four regions, and two harvest years where all samples were tested. The data was analyzed by ANOVA JMP PRO 15 (SAS Institute Inc, 2019) and means were separated by Tukey HSD with significance level set at $P < 0.05$. Results showed state was significant ($P < 0.05$) for trypsin inhibitor, protein dispersibility index (PDI), and protein solubility. While SBM supplier and region was significant for trypsin inhibitor and PDI only. For those parameters that were significant, northeastern region and states were of the highest ($P < 0.05$) for trypsin inhibitor, PDI and protein solubility. The typical benchmark of urease activity to evaluate SBM quality was not significant for supplier, region or state. However, the coefficient of variation (CV) of urease activity ranged from 31% to 70%, further indicating this parameter to be too variable to utilize as an indicator of SBM quality. Total raffinose oligosaccharides (stachyose+raffinose+verbascose) was also analyzed and indicated a significant ($P < 0.05$) difference for state, with samples from Mississippi being the lowest, supplier and region. Samples from the Midwest region had the highest ($P < 0.05$) total oligosaccharides. This study indicates the significant differences between supplier, the state in which

that supplier resides and the region the soy comes from can play a role in the quality and nutritive factors.

Key Words: Soybean Meal, antinutritive factors, trypsin inhibitor, PDI, protein solubility

M110 Determination of the standardized ileal digestible calcium requirement of fast-growing broilers from 25 to 42 days post-hatch Carrie Walk¹, Zhenzhen Wang², Shikui Wang², Jose-Otavio Sorbara¹, Jingcheng Zhang² ¹*DSM Nutritional Products*, ²*DSM Nutritional Products, Animal Nutrition Research Center*

An experiment was conducted to determine the standardized ileal digestible (SID) calcium requirement of Arbor Acres Plus male broilers from 25 to 42 days post-hatch. Broilers were obtained at hatch, placed in floor pens and fed a nutrient adequate diet until day 24 post-hatch. On day 25, 1,200 birds were weighed and allocated to one of 4 dietary treatments. There were 25 birds/pen and 12 pens/diet. The diets were formulated to contain graded concentrations of SID Ca at 0.46, 0.35, 0.24 or 0.13%. Available P (avP) was maintained at 0.39% in all diets, including 0.16% avP expected from 2,500 FYT/kg of phytase. Data were subjected to an analysis of variance and the model included SID Ca and block. Means were separated using linear and quadratic orthogonal contrasts. The SID Ca requirement was estimated using 3 different non-linear models, including quadratic, straight broken-line (SBL) and quadratic broken-line (QBL). There was no effect of graded levels of SID Ca on feed intake ($P = 0.724$), body weight gain ($P = 0.615$), feed conversion ratio ($P = 0.728$) or livability ($P = 0.391$) from day 25 to 42 post-hatch. Tibia ash percent was greatest in birds fed 0.35% SID Ca and lowest in birds fed 0.13% SID Ca (quadratic, $P = 0.063$). There was no impact of SID Ca on tibia Ca ($P = 0.312$) or P ($P = 0.223$). Apparent ileal digestibility (AID) of Ca was highest in birds fed the diets containing 0.13% SID Ca and decreased (quadratic, $P = 0.014$) as dietary SID Ca increased to 0.46%. Apparent digested Ca was highest in birds fed 0.35% SID Ca and lowest in birds fed 0.13% SID Ca (quadratic,

$P = 0.005$). Decreasing the concentration of SID Ca in the diet from 0.46 to 0.13% linearly ($P < 0.0001$) decreased ileal phytate P content by 61% and linearly ($P < 0.0001$) increased the AID of P and apparent digested P. Litter N or P were lowest in birds fed 0.35% SID Ca and increased (quadratic, $P \leq 0.05$) as dietary SID Ca decreased to 0.13% SID Ca. Non-linear equations, developed using tibia ash percent, digested Ca, litter N or litter P, estimate the SID Ca requirement of Arbor Acres Plus broilers from day 25 to 42 was 0.37, 0.35, 0.33 or 0.35%, respectively. This corresponds to an SID Ca to available P ratio of 0.95 to 0.85.

Key Words: broiler, digestible calcium, litter, phosphorus, phytate-free

M111 Feasibility in the use of purified sources of dietary fiber in the evaluation of intestinal viscosity, organ growth, intestinal morphology, and nutrient digestibility Oscar Tejada¹, Woo Kim² ¹*Southern Arkansas University, Department of Agriculture*, ²*The University of Georgia, Department of Poultry Science*

An experiment was conducted to determine the effects of soluble to insoluble fiber ratios on the intestinal viscosity, organ growth, intestinal morphology, and nutrient digestibility. A total of 432 one-day old Cobb® male broilers were randomly assigned to six dietary treatments and reared to 21 days of age in battery cages ($n=6$ replicates per treatment). A semi-purified basal diet with 0% crude fiber was formulated to which the adequate soluble to insoluble fiber ratio was added. All birds were fed a common corn-soybean meal diet during the first 7 days to ensure proper organ growth development, before being exposed to the semi-purified experimental diets. Growth performance was measured on days 14 and 21. Ileal samples were collected on days 14 and 21 for analyses of intestinal viscosity. On day 21, ileal digesta were collected from 7 birds per replicate for nutrient digestibility analyses, and one bird was used to collect sections of the duodenum, jejunum, and ileum for intestinal morphology analyses. Higher soluble to insoluble fiber ratios resulted in poorer gain per bird and feed efficiency from d 7 to 21 ($P < 0.01$). No statistical differences were observed in the organ growth among the dietary treatments ($P > 0.05$). No differences in the duodenal villus to crypt ratio were observed among the dietary treatments ($P > 0.05$). However, the group fed the lowest soluble to insoluble fiber ratio had the smallest villus to crypt ratio, and the biggest ileal villus to crypt ratio ($P < 0.001$). Decreases in digestibility of dry matter, apparent metabolizable energy and crude protein were observed in increases in the soluble to insoluble fiber ratio ($P < 0.05$). No statistical differences in intestinal viscosity were observed from d 7 to 14 ($P > 0.05$). However, higher soluble to insoluble fiber ratios resulted in higher intestinal viscosity on day 21 ($P = 0.09$). In conclusion, purified sources of dietary fibers may not be adequate in the evaluation of digestive organ growth and intestinal morphology but can be used to evaluate the impact of fibers on the nutrient digestibility.

Key Words: dietary fiber, soluble fiber, insoluble fiber, nutrient digestibility, intestinal viscosity

M112 Enzymatically treated yeast to affect gut integrity and growth performances of Eimeria challenged broiler. Hagen Schulze¹, Douglas Haese² ¹*Livalta / AB Agri*, ²*Centro de Tecnologia Animal*

Eimeria is known to cause coccidiosis, an endemic disease, that has been estimated to induce losses of US\$ 0.17/ slaughtered chicken in North America. The aim of this study was to evaluate an enzymatically treated yeast (LivaltaCell HY40, Livalta, UK; HY40) on gut integrity and growth performance of *Eimeria* sp. challenged broiler chickens. Eighty-four groups of 30 male broiler chicks (Cobb 500) were allocated to one of 7 dietary treatments, containing either 0, 0.05, 0.2, 0.8 or 3.2% HY40 (treats C, D, E, F, G), formulated in corn and soybean meal- based diets in four phases: 1 (d 0-7), 2 (d 8-21), 3 (d 22-33) and 4 (d 34-42). Treats A and B contained coccidiostats in phases 1, 2 and 3. On d 14, birds in treats B to G were challenged with a blend of *Eimeria tenella* (2,000 oocyst/bird), *E. acervulina* (25,000 oocyst/bird) and *E. maxima* (10,000 oocyst/bird)

by oral gavage, whilst birds of treat A received a saline solution. Growth performance parameters (body weight, BW; feed intake; feed conversion ratio, FCR) were recorded on d 7, 21, 33 and 42. Jejunal and caecal digesta oocysts counts (OC) were measured on d 21 and 42. Birds were scored for lesions (LS) at duodenum for *E. acervulina*, jejunum for *E. maxima* and ceca for *E. tenella* at d 21. Lowest OCs were found throughout for treat A and no treat effects on d 42. On d 21, treat B effectively reduced ($P < 0.05$) jejunal OC compared with treat C. In birds of treat E, F and G caecal OCs were reduced ($P < 0.05$) compared with treat D. Birds of treats A and B had minimal gut lesions (LS 0 and 7) whereas treats C and D had highest LS (33 and 33). Starting from 0.2% HY40, treat E, F and G, LS was strongly reduced with 12, 13 and 11, respectively, compared with treat C and D. There was no differences ($P > 0.05$) on growth performance between birds of treats A and B. Overall, birds of treat C had significantly reduced BW (-226g) and FCR (+7 points) compared with treat A. Whilst starting from 0.2% inclusion, treat E, F and G, HY40 significantly improved d 42 BW by 4% (+101g) and FCR by -6 points, reaching comparable FCR to birds treated with coccidiostat (treat A and B). In conclusion, HY40 can be an effective ingredient in poultry antibiotic-/ coccidiostat-free feeding systems to support welfare and performance of the birds.

Key Words: Enzymatically treated yeast, Performance, Eimeria challenge, Oocyst count, Broiler

M113 Histological and gene expression analyses of broiler jejunal and liver tissues from commercial farms in relation to flock performance.

2. Gene expression Luis Romero¹, Davis Fenster², Candice Blue², Mislav Acman¹, Don Ritter³, Rami Dalloul² ¹*anh-innovation Lda.*, ²*University of Georgia*, ³*Poultry Business Solutions LLC*

Factors causing sub-optimal performance are often subtle and difficult to pinpoint. This study used transcriptomics data from chicken tissues to understand differences between broiler flocks differing in final performance. Four flocks of straight run Ross-708 broilers of the same age were selected from farms with a wide variation of recent historic FCR in a commercial broiler integration. Flocks were on a NAE, all-vegetable diet program and received a coccidiosis vaccine at hatch. At 28 d, 16 healthy, randomly selected birds per farm were euthanized. A section of liver was sampled and RNA was extracted using the Zymo Direct-zol RNA kit with TRI reagent. mRNA was sequenced using the Illumina platform with PolyA selection to yield >20M pair-end reads per sample. Gene expression abundances were calculated against the reference chicken genome. Differential gene expression between High (Farm 2 and 3) and Low (Farm 1 and 4) performing farms were analyzed using the DEseq package of R, and the enrichment of pathways from the Reactome database were evaluated using ClusterProfiler. Final FCR were 1.951, 1.901, 1.914, and 1.923 and final BW were 4.188, 4.246, 4.309, 4.096 kg/bird for Farms 1 to 4, respectively. Low performing farms had 1,308 differentially expressed genes (adj $P < 0.05$) versus High performing farms. Low performing farms had 22 pathways downregulated ($q < 0.05$) compared to High performing farms, including 11 pathways related to energy metabolism and 6 pathways related to the metabolism of amino acids such as tryptophan, sulfur- and branched-chain amino acids. Low performing farms had 42 pathways upregulated ($q < 0.05$), including 9 related to signal transduction, 7 immunity related such as IFN signalling, and 6 related to cellular response to stimuli, particularly heat stress. Differences in gene expression and pathway enrichment corresponded to a difference ($p < 0.01$) in the jejunal villi length: crypt depth ratio between Low (3.36) and High (4.57) performing farms. However, no differences were detected by macroscopic observation at necropsy. Transcriptomics data from commercial broiler farms provided additional insights into the health and nutrition of broiler chickens, which can inform decisions to enable reaching their genetic potential.

Key Words: broiler, gene expression, liver, performance, jejunum

M114 Effect of feeding mango seed kernel on performance and carcass characteristics of broilers Tuba Riaz¹, Zafar Hayat^{1,2}, Abdur Rahman¹, Mazhar Abbas¹ ¹*CVAS-Jhang Campus, University of Veterinary and Animal Sciences*, ²*Department of Animal Sciences, College of Agriculture, University of Sargodha*

The conventional feed resources for poultry production are mainly cereal based which competes with human food. As the feed cost accounts for up to 70% of total cost of poultry production and remains the greatest constraint there is need to cope with the feed cost issue by utilization of non-conventional feed resources in broiler feed which could be the by-products coming from industries, fruits and vegetables waste. Considering mango waste, consisting of peel and seed, as a potential one among all resources which is almost half of its total production can serve this purpose. Mango seed kernel (MSK) is considered to be a good replacement of maize in broiler due to its nutritional profile that is capable to provide as much energy as maize can. So the study was designed to evaluate the effects of MSK by replacing maize levels on the performance, carcass characteristics in broilers and the economics of production. By following completely randomized design, 300 broiler chicks (day old, Cobb-500) were distributed into 4 dietary treatment groups with 5 replicates, each

group containing 75 birds. The experimental diets were formulated to contain 0%, 5%, 10% and 15% of MSK as replacement of maize inclusion levels, respectively. Statistical analysis of the results showed non-significant ($p>0.05$) differences in overall feed intake of birds among all treatments during experiment. While significant differences ($p<0.05$) were observed in body weight gain and FCR of birds among treatments having MSK up to 10% as maize replacement during all phases. Inclusion of MSK in diet had no significant effect on carcass characteristics as well as giblets weights of the birds between treatments. Economics evaluation of trial revealed that replacement of maize by MSK had positive effects on economics of broiler production and by replacing maize with MSK up to 10% level had almost similar results in terms of return cost and feed cost/kg weight gain as compared with control group while feed cost/kg in other treatments was reduced as the level of MSK in the diet was increased. Based on results, it can be concluded that maize can be replaced with MSK up to 10% inclusion level in broilers diet without affecting the growth performance of birds as well as for economical broiler production.

Key Words: mango seed kernel, broilers, non-conventional feed ingredient

Environment, Management and Animal Well-Being: Environmental Impacts

M115 Optimization of a loop-mediated isothermal amplification (LAMP) assay for the detection of *Clostridium perfringens* Deepa Chaudhary^{*1GS}, Linan Jia¹, Anuraj Sukumaran¹, Wen-Hsing Cheng², Aaron Kiess³, Li Zhang¹ ¹*Department of Poultry Science, Mississippi State University*, ²*Department of Food Science, Nutrition and Health Promotion, Mississippi State University*, ³*Prestage Department of Poultry Science, Carolina State University*

Clostridium perfringens (*C. perfringens*) causes necrotic enteritis, an economically significant poultry disease that accounts for over \$6 billion in annual economic losses on a global scale. Therefore, rapid detection for *C. perfringens* is essential for the surveillance and assessment of pathogen spreading. The loop mediated isothermal amplification (LAMP) assay is a new technology for rapid, sensitive, and specific detection of pathogens and has potential to be performed on-site. In this study, two sets of LAMP primers ID7 and ID22 comprising forward and reverse inner primers (FIP and BIP) and outer primers (F3 and B3) targeting *C. perfringens* *cpa* gene were designed. LAMP assay was performed with Warmstart Colorimetric LAMP 2X master mix and the results were visualized by color change and verified by agarose gel electrophoresis. The LAMP assays were optimized for primer concentration, reaction temperature and time. Inclusivity tests were conducted using 7 *C. perfringens* strains; exclusivity tests were conducted using 6 non-*C. perfringens* (*Clostridium* species) strains and 17 non-*Clostridium* strains (*E. coli*, *Campylobacter*, and *Salmonella*). Sensitivity was tested with serially diluted genomic DNA of *C. perfringens* ATCC 13124 and compared with PCR results. The optimized primer concentrations were 1.2 mM FIP/BIP and 0.2 mM F3/B3. The LAMP reactions were carried out at 63°C for 30 mins. The inclusivity of the LAMP assays with both primer sets was 100%. The exclusivity of LAMP assays with both primer sets for non-*C. perfringens* isolates, and non-*Clostridium* isolates were 88.33% and 100%, respectively. The specificity of LAMP assays for the detection of *C. perfringens* was consistent with PCR. The detection limit for the LAMP assays with ID7 and ID22 primer set, and for PCR was 2 pg/μL, 0.2 pg/μL and 2 pg/μL, respectively. LAMP assay with ID22 showed excellent sensitivity and was 10 times more sensitive than PCR. This study identified suitable primer set and optimized the reaction conditions for developing a potential rapid detection tool for *C. perfringens* to minimize the spreading of this pathogen in poultry farm.

Key Words: *Clostridium perfringens*, necrotic enteritis, rapid detection, LAMP assay, PCR

M116 Genotypic-phenotypic discrepancies between antimicrobial resistance characteristics of *Escherichia coli* isolated from asymptomatic and diseased broilers Priyanka Devkota^{*1GS}, Sabin Poudel¹, Xue Zhang², Anuraj Sukumaran¹, Pratima Adhikari¹, Reshma Ramachandran¹, Aaron Kiess³, Li Zhang¹ ¹*Department of Poultry Science, Mississippi State University*, ²*Department of Food Science, Nutrition and Health Promotion, Mississippi State University*, ³*Prestage Department of Poultry Science, North Carolina State University*

Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis, resulting in significant economic loss to the poultry industry. The control of APEC infections relies on antibiotic treatment; however, the constraint of this disease control strategy is the emergence of resistant strains. This study aimed to investigate the genotypic and phenotypic antimicrobial-resistance profiles of the *E. coli* strains isolated from broilers. A total of 66 *E. coli* were isolated from broiler farms, including 22 isolates from asymptomatic carrier birds and 44 isolates from organs of birds with systemic infection. Polymerase chain reactions were conducted to detect 13 antimicrobial resistance genes in these *E. coli* isolates. Antimicrobial susceptibility of *E. coli* isolates was tested by the broth microdilution method using a Sensititre NARMS gram-negative panel. Statistical analysis was performed by the Chi-square test using the SAS 9.4 software. Antimicrobial susceptibility testing showed a difference in streptomycin (79.5%^a vs. 31.8%^b) and azithromycin (0%^b vs. 13.6%^a) resistance among disease-associated and asymptomatic carrier strains ($P < 0.05$). High percentages of resistance were observed in disease-associated and asymptomatic carrier strains for tetracycline (93.2% vs. 95.5%) and gentamicin (54.5% vs. 40.9%). The genotypic results showed a high prevalence of tetracycline gene *tetA* among disease-associated isolates (75.0%) and asymptomatic carrier isolates (59.1%) respectively. In addition, the asymptomatic carrier strains had a higher prevalence of the *bla*_{TEM} gene compare with disease-associated strains (31.8%^a vs. 6.8%^b, $P < 0.05$). Out of 66 *E. coli* isolates, 44 (66.7%) were multidrug resistant. The data revealed that APEC isolates possessed a higher percentage of phenotypic and genotypic resistance to diverse antimicrobials and possess a higher rate of multidrug resistance. This study helps to understand the epidemiological background of drug-resistant *E. coli*. In summary, a surge of antimicrobial resistance compro-

mises the effective long-term APEC treatment, making the development of alternative strategic approaches to control APEC essential.

Key Words: *Escherichia coli*, antimicrobial resistance genes, antimicrobial susceptibility testing, genotype and phenotype, multidrug resistance

M117 Characterization of virulence-associated genes and phylogenetic groups of Avian pathogenic *Escherichia coli* isolated from broiler breeders with colibacillosis from Mississippi Madalyn Jennings^{*UG}, Li Zhang, Pratima Adhikari, Reshma Ramachandran *Mississippi State University*

Avian pathogenic *Escherichia coli* (APEC) is a diverse pathogen causing colibacillosis in poultry. Colibacillosis infection can cause symptoms such as septicemia, perihepatitis, salpingitis, airsacculitis, and pericarditis. It is one of the most detrimental diseases to the poultry industry due to its high morbidity and mortality rates that result in economic burden. The disease-causing ability of APEC depends on multiple virulence factors as well as host factors, and so far, no single virulence gene has been specifically associated with APEC pathogenicity. Hence, the virulence characterization of APEC isolates is imperative in understanding the pathogenesis of colibacillosis and to develop effective intervention strategies to control APEC infection. Thus, the objective of this study was to determine the occurrence of virulence-associated genes and the phylogenetic groups of APEC strains isolated from broiler breeders with symptoms of colibacillosis presented at the Mississippi Poultry Research and Diagnostic Laboratory. A total of twenty-eight APEC isolates recovered in MacConkey agar and further confirmed by real-time PCR for *ybbW* gene were used. Further, bacterial genomic DNA was extracted, and PCR-based method was used for the detection of ten virulence-associated genes and the phylogenetic groups of APEC isolates. Of the twenty-eight isolates screened, 96.43% of the isolates harbored at least one virulence-associated gene. The genes encoding for iron acquisition (*iroN* and *iutA*), protectins (*iss* and *ompT*), and toxin production (*hlyF*) exhibited the highest prevalence (69 to 78.6%), followed by adhesins (*papC* and *tsh*; > 39%), invasins (*ibeA*; 14.30%), and colicin V plasmid operon (*cva/cvi*; 28.60%). Further, majority of the isolates belonged to the B2 phylogroup (71.43%), followed by D phylogroup (25%), and only one isolate represented B1 phylogroup (3.60%). In conclusion, this study shows that APEC strains isolated from diseased broiler breeders in Mississippi harbors a variety of virulence-associated genes and mainly belong to the phylogenetic groups representing virulent extraintestinal *E. coli*. Further studies on the virulence profiles of APEC are still required to confirm the pathogenesis and to develop effective intervention strategies to control APEC.

Key Words: *Escherichia coli*, colibacillosis, virulence genes, phylogenetic groups, broiler breeders

M118 Effects of pullets age and activities on air quality in the cage-free house Ramesh Bist^{*IGS}, Xiao Yang¹, Sachin Subedi¹, Yangyang Guo¹, Milan Sharma¹, Amit Singh¹, Woo Kim¹, Casey Ritz¹, Deana Jones², Prafulla Regmi¹, Lilong Chai¹ *¹Department of Poultry Science, University of Georgia, ²US National Poultry Research Center, USDA ARS*

Due to concern on welfare of laying hens in conventional cages, the primary food chains and grocers have pledged to only source cage-free (CF) eggs by 2025. As compared to conventional cage systems, an inherent challenge with CF housing is the poor indoor air quality, i.e., high ammonia (NH₃), particulate matter (PM) and airborne bacteria (AB) levels. Studies have been conducted on commercial cage-free farms during lay period, but limited studies investigated the air quality during pullet phase. The objectives of this study were to examine the effect of pullets' age and activities on NH₃ and PM levels in the CF housing system. A total of 800 Hy-line W-36 commercial day-old chicks were randomly allocated into four identical rooms, which were assigned with similar feed, water, lighting, and bedding or litter. Indoor temperature and ventilation control

were identical for all rooms. The dust data were collected twice a week from the first week of age (WOA) at three different locations (i.e., near perch, between feeder and drinker, and near exhaust fan) of each room by using TSI Dusttrak 8533. Ammonia concentrations were monitored with DOL-53 sensors and Onset HOBO RX3000. In this study, the dust and NH₃ information collected until 11-WOA were analyzed. The air quality in different rooms and changes over time were analyzed using JMP pro-16 by LSmeans Tukey HSD. The difference was considered significant when $p < 0.05$. The dust of PM_{2.5}, PM₁₀, and total suspended particles (TSP) increased significantly as birds age from 1 to 11 WOA (e.g., PM_{2.5} and TSP levels were 0.023 ± 0.003 and 0.059 ± 0.012 mg/m³ on the first week, but the levels reached 0.596 ± 0.22 mg/m³ and 2.440 ± 0.477 mg/m³ by 11 WOA, respectively) ($p < 0.001$). In addition, dust levels measured near perches were three time higher than that measured between feeder and drinker or near the exhaust fan ($p < 0.001$). The significant interaction between the age of pullets and dust levels was developed ($p < 0.001$). For NH₃ levels, the average daily concentration was <1 ppm as of 11 WOA for all rooms due to dry litter conditions (i.e., 9-10% litter moisture content). Our findings will provide basis for air quality management for improving health and welfare of pullets in cage-free facilities.

Key Words: egg production, cage-free system, pullets, dust, ammonia

M119 Campylobacter prevalence differs across and within broiler houses with re-used poultry litter Reed Woyda^{*IGS}, Adelumola Oladeinde², Dinku Endale³, Timothy Strickland³, Babafela Awosile⁴, Zaid Abdo¹ *¹Colorado State University, Program in Cell and Molecular Biology, ²Egg safety and Quality Research Unit, US National Poultry Research Center, USDA, ³Southeast Watershed Research, USDA, ⁴School of Veterinary Medicine, Texas Tech University*

Multiple hurdle interventions are recommended during the processing of broiler carcasses to reduce bacterial loads, but pathogens like *Campylobacter* remain a major foodborne disease linked to poultry consumption. Consequently, the poultry industry and regulatory agencies are looking for pre-harvest strategies that can reduce *Campylobacter* in broiler grow-out houses. To achieve this aim, it is critical we understand the ecology of *Campylobacter* in pre-harvest. In this study, a longitudinal sampling of the litter in four commercial broiler houses was conducted over three consecutive flocks to evaluate *Campylobacter* prevalence in litter. Prior to the start of the study, a complete house clean-out was done, and fresh peanut hull was used as the bedding for the first flock. The second and third flock were raised in succession on the same litter without any litter clean-out between each grow-out cycle. Litter was sampled at the beginning of each grow-out cycle and at the end of the cycle. Each house was divided into four sections: front, mid-front, mid-back, and back. For each of these sections, three grab samples of litter were collected and pooled. Seventy-two pooled litter samples were taken from each house, totaling 288 litter samples across all houses. *Campylobacter* was detected from litter samples by direct plating and enrichment. Statistical analysis was used to explore the relationship between *Campylobacter* prevalence and relevant physio-chemical parameters. *Campylobacter* was found to be most prevalent during the first flock and in houses 3 and 4. Across all houses the front section was most likely to harbor *Campylobacter*. Early grow-out period had significantly less *Campylobacter* than the late grow-out period. Furthermore, the odds of finding *Campylobacter* increased when the house temperature was below or equal to 79°F than at temperatures higher or equal to 80°F. We also observed that higher litter moisture content (>25%) resulted in higher *Campylobacter* prevalence. Lastly, genetic analysis of isolates via whole genome sequencing revealed that *C. jejuni* was the dominant species in the litter (39/44); *C. coli* (5/44). This study suggest that interventions to decrease litter moisture and grow-out house temperature control may reduce *Campylobacter* prevalence.

Key Words: *Campylobacter*, litter-reuse, peanut-hulls, broilers, pathogens

M120 Evaluation of broiler performance fed nicarbazin: RH Effect
Kristin Miles^{*GS}, Michael Czarick, Garret Ashabrunner, Brian Fairchild
University of Georgia

The anticoccidial, nicarbazin, is known to cause a reduction in performance and an impairment in the ability to cope with high ambient air temperatures. It is well established that heat stress can be intensified by increased relative humidity (RH). The purpose of this study was to determine the effect of nicarbazin fed for 28 days on performance of broilers at two levels of RH: 35-50% and 65-75%. 864 Ross 708 male chicks were assigned to one of four treatment groups: 30-50% RH, fed a control diet, 30-50% RH, fed a nicarbazin diet, 65-75% RH, fed a control diet, and 65-75% RH, fed a nicarbazin diet. The birds were reared for 35 days during which the nicarbazin diet treatment was fed for the first 28 days then the birds were switched to control diet for remainder of the study. The treatment diet contained nicarbazin at a rate of 125 ppm and 99 ppm for the starter and grower feeds respectively while control diet did not contain nicarbazin. A coccidiosis challenge was not administered for this study and birds were necropsied at 21 days and evaluated for coccidiosis. No evidence of coccidiosis was found. Data were analyzed via JMP Pro 15, using a split plot ANOVA while separating means with Student's *t* ($P < 0.05$). Nicarbazin treated birds had significantly lower body weights and feed consumption than those fed the control diet for all five weeks ($P < 0.05$). FCR was significantly increased in the nicarbazin treated broilers compared to the birds fed the control diet. There was a significant decrease in weight gain for the nicarbazin treatment group for the first four weeks but was no longer significant after nicarbazin was removed from the treatment diet ($P < 0.05$). No significant differences were seen for weight, gain, and feed consumption between the RH treatments or the interactions between RH and diet. The interaction between RH and diet showed a significant difference in FCR for Weeks 3 and 4 ($P < 0.05$). Treating with nicarbazin caused a decrease in overall broiler performance and higher RH level in combination with nicarbazin treatment was associated with higher FCR. Another trial is planned to confirm these results.

Key Words: anticoccidial, relative humidity, feed conversion

M121 Evaluating the effect of day length (24 vs 20 hours) during brooding on broiler performance
Garret Ashabrunner^{*GS}, Michael Czarick, Kristin Miles, Brian Fairchild
University of Georgia

Dark periods are well documented to be beneficial for broiler performance, health, and welfare. Conventionally, broiler chicks are initially grown with a limited (one hour) or no dark period in hope that they will consume more feed and water over the course of a day. With several studies documenting the benefits of a dark period after brooding, it is questionable how birds would benefit from a dark period during brooding. Two preliminary trials were conducted to explore if providing chicks with 24 hours of light affects early flock performance compared to providing chicks with 20 hours of light. Four pens of 20 Cobb500 male chicks were placed in six identical rooms (three rooms per treatment). The treatment chicks were provided 20 hours of light while control given 24 hours of light. Trial two utilized a 30-minute sun rise/set function where Trial one had lights turn on and off. At seven days of age both treatments were given 20 hours of light. Birds were weighed and feed consumption calculated on Days 3, 7, 10, and 14. All data were analyzed using the JMP Pro 15 ANOVA procedure ($p < 0.05$). The mean of each room was taken and analyzed against each other by treatment to account for room effects, creating a sample size of three per treatment. In Trial one Day 3 body weight, gain, feed consumption and Day 7 body weight were significantly higher ($p < 0.05$) for the control group. No other significant differences were found in Trial one. In Trial two no statistical differences were found between treatment and control groups in weight gain, feed consumed, and feed conversion through Day's 3, 7, 10, and 14 ($p < 0.05$). In both trials a trend of higher body weight and feed consumption in days 3 to 7 was observed in the control birds. This trend reversed after the control was given a dark period. The results of these preliminary trials suggest that giving broiler chicks a four-hour dark

period for the first seven days of a flock does not affect performance at 14 days of age. More trials will need to be conducted to see the how levels of daylength during brooding affect broiler performance, health, and welfare.

Key Words: Chick management, lighting, dark period

M122 Assessment of early-stage thermal variation on broiler chicken embryonic mortality and hatchability
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With improvements in broiler performance, interest in the impact of thermal manipulation (TM) during critical periods of broiler embryogenesis has been renewed. Timing and duration of TM can alter egg moisture loss, embryonic mortality, hatchability, and chick BW. The objective was to evaluate the effect of TM during early-stage incubation on hatch characteristics and chick BW. Ross 708 × Yield Plus broiler breeder eggs ($n = 7,200$) were weighed and sorted by 1-g increments at 18.3 °C. Eggs (total $n = 2,160$) ranging from 54 to 59 g were allotted equally by weight into 90-egg trays ($n = 4$ trays per incubator). All eggs were pre-warmed at 24 °C for 8 h and incubated at 37.5 °C and 65% RH from embryonic day (ED) 0 to 4. From ED 4 to 11, eggs were incubated at 1 of 3 temperatures ($n = 2$ incubators per TM treatment); 37.5 °C (CTL), 36.4 °C (COLD), or 38.6 °C (HOT). On ED11, all incubators were returned to the CTL (37.5 °C) set point until ED 18. On ED18, all eggs were individually weighed to determine moisture loss and transferred to baskets in hatchers set to 36.7 °C and 60% RH. After 514 h of incubation, all chicks from all hatchers were removed simultaneously, vent sexed, and weighed. Embryonic mortality (total dead; early, middle, late-dead; pipped; malpositioned) was assessed on all unhatched eggs 6 h after chick processing. Data were analyzed as a one-way (TM) or two-way (TM × sex) ANOVA with the GLIMMIX procedure of SAS. MS were separated at $P \leq 0.05$ with the PDIF option. Pre-incubation egg weights were similar among all treatments ($P = 0.4648$). As expected, eggs from HOT incubators were lighter than those from COLD incubators and similar in weight to those incubated at the CTL temperature ($P = 0.0442$). No differences were observed in any embryonic mortality category assessed ($P \leq 0.5921$). A 1.1 °C temperature gradient during early-stage incubation (ED 4 to 11) did not alter embryonic mortality, proportions of male and female chicks hatched ($P \geq 0.1332$), or chick BW at hatch ($P \leq 0.2897$). For chick BW at hatch, no temperature treatment by chick sex interaction was observed ($P = 0.7387$). Though COLD chicks were heavier at hatch than HOT and CTL chicks ($P = 0.001$), this is likely due to differences in hatch pattern among TM treatments.

Key Words: broiler chicken, incubation, embryonic mortality, thermal manipulation, hatchability

M123 Effect of temperature, relative humidity, and thermal humidity index on live performance parameters of broilers raised under commercial tropical conditions
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Although temperature (T) and relative humidity (RH) are usually monitored in broiler farms, data about the impact of these variables under commercial operations are still scarce. This study aimed to evaluate the effect of T, RH, and a thermal humidity index (THI) on BW, BW gain, feed conversion ratio (FCR), and mortality of broilers raised to 35 d under commercial tropical conditions. The analyzed data included performance records of 38 male and 42 female Ross 308 AP broiler flocks subject to continuous environmental monitoring between January and July of 2020. Data were analyzed using R software in RStudio. Hours of exposure were calculated for each flock from the placement up to 35 d at T lower or greater than 1.5, 2.5, 5.0, and 7.0 °C from the Aviagen chick management

recommendations. Time in RH below 50 and above 75% was also calculated. Additionally, a THI was computed based on the equation described by Berman *et al.* (2016). Daily THI boundaries were determined as the combination of recommended T and RH. Correlation analyses were conducted, and linear models fit between live performance parameters and hours of exposure to non-recommended T, RH, and THI levels. Negative correlations of BW ($P<0.05$) were detected when chickens were exposed to T 5 °C below recommendations at 7, 14, and 21 d. Linear regression estimates indicated ($P<0.05$) that chickens were lighter 1.51, 3.50, and 4.91 g, respectively, for each additional hour they were exposed to these conditions. Also, FCR at the second and third wk of age were affected ($P<0.05$) by cooler environmental T. Mortality increased at 21 and 28d ($P<0.05$) 0.01% per each additional hour of exposure to T above 7 °C

from 7 to 14 d. Correlation coefficients from RH were between 0.41 and 0.51, while regression analyses resulted in poor goodness-of-fit. The THI was only correlated to FCR at 21 d ($P=0.025$; $r = 0.69$). In conclusion, chickens' BW, BW gain, and FCR were more affected by low T during the first three wk, while mortality was related to exposure of 7 °C higher than recommended. RH seemed to be a controlled parameter that did not impact the live performance of chickens, while the THI did not exhibit the effects observed in T. Then, its use could not reveal the actual effects of the environment in commercial farms.

Key Words: Data analytics, environment, temperature, live performance, relative humidity

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

M124 Evaluating growth performance, intestinal barrier integrity, and meat quality of random bred broiler populations and a common ancestor under heat stress conditions. Travis Tabler^{GS}, Elizabeth Greene, Nicholas Anthony, Sami Dridi, Sara Orlowski *University of Arkansas*

Heat stress (HS) has a known negative effect on poultry performance and production sustainability due to the adverse impact it has on bird welfare, health, and growth. Although modern commercial broilers have increased gastrointestinal mass and higher feed efficiency than unselected birds, they are more susceptible to HS induced "leaky gut syndrome," or increased gastrointestinal permeability. The aim of the study was to observe the effect of HS on growth performance, intestinal barrier integrity, and processing performance in three modern broiler lines and their ancestor Wild Jungle Fowl. Four chicken populations including Giant Jungle Fowl (JF), Athens Canadian Random Bred (ACRB), 1995 Arkansas Random Bred (95RAN), and 2015 Modern Random Bred (MRB) were studied. Day-old male broiler chicks ($n = 150/\text{line}$) were placed by line in environmentally controlled chambers and raised under thermoneutral (TN) conditions with feed intake, water intake, and temperature measured daily; body weight gain measured weekly. On day 28 the birds were subjected to one of two environmental conditions: TN (24°C) or chronic cyclic HS (8-h a day at 36°C) and grown out until processing following day 54. Growth performance, metabolite concentrations, and processing data were analyzed by two-way ANOVA. These data shown the significant effect HS had on growth performance, intestinal barrier integrity, and meat quality of the studied modern broilers. Based on the results of this study, it appears that HS has a greater negative impact on the higher yielding commercial lines (MRB and 95RAN) than the ACRB and JF, delineating the clear line differences that exist between the random bred lines and their common ancestor.

Key Words: Heat Stress, Gut Health, Broiler, Processing, Genetics

M125 Evaluation of head removal following cervical dislocation for broiler euthanasia Alexandra Jackson^{UG}, Andrea Urrutia, Leticia Orellana Galindo, Marco Reina, Montana Riggs, Dianna Bourassa, Bethany Baker-Cook *Auburn University*

Whether the occurrence of accidental head removal with cervical dislocation impacts broiler welfare during euthanasia has been called into question. This study aimed to evaluate the effect of head removal with cervical dislocation on the time to insensibility and / or death (via the postmortem response) of broilers who had their head removed after cervical dislocation versus broilers that did not. Sixty 50 d of age broilers (30 male and 30 female; mean weight: 3.7kg) were randomly selected and assigned to either the control treatment (cervical dislocation only)

or the head removal treatment (cervical dislocation and head removal). Each bird was euthanized by cervical dislocation prior to the assessment of postmortem responses. The responses assessed indicate bird sensibility (loss of consciousness and absence of pain) and death, both important for euthanasia in bird welfare. For the head removal treatment, the neck was severed through the gap formed in the spine from cervical dislocation within 10 s following euthanasia. The nictitating membrane reflex was tested at 15 s intervals post-euthanasia, to measure time to insensibility and was recorded along with time of cessation of bird movement, to measure time to death. Statistical analysis was performed using ANOVA with a significance at $P \leq 0.05$. The main effects evaluated were sex, treatment, and sex*treatment interactions. The nictitating eye reflex was only observed in two birds within the control group (was lost within 30 seconds). No significant differences in time to cessation of movement were found for sex ($P=0.6471$), treatment ($P=0.1503$), or sex*treatment interactions ($P=0.1552$). The average time of postmortem response of movement was 131 s for male broilers and 126 s for female broilers. The average time of postmortem response of movement was 135 s in the control group and 122 s for the head removal treatment. Overall, the study results suggest that there was no effect on bird welfare as no difference was found for time to insensibility (nictitating eye reflex) and time to death (duration of movement) due to head removal following euthanasia by cervical dislocation. It can be concluded that accidental head removal during broiler euthanasia by cervical dislocation may not be a bird welfare issue.

Key Words: euthanasia, broiler, cervical dislocation

M126 Effect of silvopasture versus open pasture systems on fearfulness and leg health in fast-growing broiler chickens Bidur Paneru^{GS}, Shawna Nastasi, Gabriel Pent, Adam Downing, John Munsell, John Fike, Leonie Jacobs *Virginia Polytechnic Institute and State University*

A silvopasture system involves the deliberate integration of trees, forages, and livestock, which may result in high-quality habitat for broiler chickens. However, such systems have not been widely adopted by the poultry industry. The aim was to investigate the impact of silvopasture (SP) or open pasture (OP) access on fearfulness and leg health in fast-growing broilers. A total of 886 day-old Ross 708 chicks in Experiment 1 (E1) and 648 chicks in Experiment 2 (E2) were raised indoors from day (d) 1 until d22 (E1) or d23 (E2). Thereafter, birds were raised in SP plots (32% mean canopy cover) and OP plots (0% canopy cover). Experimental units (16 in E1 and 12 in E2) within each system (125m²) were established with flexnet electric fences and contained a chicken coop. Stocking density was 20.8 kg/m² in E1 and 21.8 kg/m² in E2 on d42-43. Fearfulness was assessed using a tonic immobility test ($n=159$ birds in E1, and $n=156$ in E2), leg health was assessed through footpad dermatitis (FPD; 0-4 score), hock burns (0-4 score; $n=160$ in E1, and $n=155$ in E2), gait (0-2 score; $n=160$

in E1, and $n=156$ in E2), and a latency-to-leave (LTL) test ($n=111$ in E1, and $n=154$ in E2) at 5 weeks of age. Data were analyzed with JMP Pro using non-parametric tests. SP birds were less fearful than OP birds (79.1 ± 8.7 vs 103.5 ± 10.0 sec; $p=0.031$ in E1, and 59.1 ± 6.7 vs 102.8 ± 9.7 sec; $p<0.001$ in E2). SP birds showed better FPD scores than OP birds (mean \pm SEM score of 0.08 ± 0.04 vs 0.19 ± 0.04 ; $p=0.012$ in E1 and 0.17 ± 0.04 vs 0.48 ± 0.08 ; $p=0.004$ in E2). SP birds had ($p=0.049$) healthier hocks than OP birds in E1 (mean score of 0.15 ± 0.04 vs 0.26 ± 0.05). In E2, SP birds tended ($p=0.074$) to show healthier hocks than OP birds (mean score of 0.03 ± 0.01 vs 0.09 ± 0.02). In E1 ($p=0.019$), but not in E2 ($p=0.217$), SP birds had worse gait than OP birds (mean score of 0.21 ± 0.04 vs 0.08 ± 0.02). LTL did not differ in E1 ($p=0.221$) or E2 ($p=0.658$). Even though gait was worsened in SP in E1, raising birds in silvopastures reduced fear and improved footpad and hock health compared to the birds raised in open pastures. Overall, both silvopasture and open pasture systems resulted in excellent leg health.

Key Words: Outdoor access, broiler chicken, fearfulness, leg health, animal welfare

M127 The effect of environmental complexity and stocking density on broiler chicken behavior Lauren Evans^{UG}, Mallory Anderson, Andrew Campbell, Lacey Daulton, Kristin Omans, Alexandra Ulans, Leonie Jacobs *Virginia Polytechnic Institute and State University*

Broiler chickens are housed in relatively barren conditions at high stocking densities, potentially contributing to low activity levels and limited expression of natural behaviors. The objective was to evaluate the impact of a complex environment and stocking density on broiler behaviors. Eight pens contained an environmental complexity (high complexity [HC] or low complexity [LC]) and stocking density (high [HD] of 12.4 birds/m² or low [LD] of 6.2 birds/m²) treatment, resulting in HC/HD, HC/LD, LC/HD, and LC/LD (HD=180, LD=90 birds/pen). Focal-animal sampling (12 birds/pen/time point) was used to record frequency and duration of behaviors continuously for 5 min at 8AM and 4PM, 1 day/week, in weeks 2, 4, and 7. The frequency (%) was calculated from total behaviors observed per pen, per time point. The duration (seconds[s]) was calculated as a sum of all bouts per pen, per time point (max 300s). Data were analyzed using Wilcoxon chi-square tests.

Birds in HC pens were inactive less often (HC=45.4 \pm 1.7%, LC=54.3 \pm 1.5%; $p<0.001$) for shorter periods of time (HC=173.0 \pm 6.6s, LC=220.2 \pm 5.3s; $p<0.001$), and ate less often (HC=3.9 \pm 0.7%, LC=4.1 \pm 0.6%; $p=0.0785$) and for shorter periods (HC=12.9 \pm 2.6s, LC=21.7 \pm 3.3s; $p=0.026$) compared to birds in LC pens. Birds in HC pens perched more (HC=8.1 \pm 1.5%, LC=0.1 \pm 0.1%; $p<0.001$) and longer (HC=32.6 \pm 5.4s, LC=1.2 \pm 0.9s; $p<0.001$), interacted with enrichments more (HC=0.4 \pm 0.2%, LC=0 \pm 0%; $p=0.002$) and longer (HC=2.1 \pm 1.0s, LC=0 \pm 0s; $p=0.003$), and tended to forage longer (HC=14.2 \pm 2.4s, LC=7.2 \pm 1.5s; $p=0.072$) than birds in LC pens. Birds in HD pens preened more frequently (HD=10.5 \pm 1.0%, LD=8.1 \pm 0.8%; $p=0.049$), foraged less frequently (HD=4.2 \pm 0.6%, LD=7.1 \pm 0.7%; $p=0.001$) and for shorter periods (HD=6.9 \pm 1.6s, LD=14.6 \pm 2.3s; $p=0.001$) than birds from LD pens. Play, locomotion, dustbathing, drinking, and resting were unaffected by treatments.

Our results suggest that housing broilers in a complex environment resulted in a more desirable behavioral repertoire with more time spent on natural behaviors such as perching and exploring enrichments, improving their welfare status compared to birds in more barren conditions. Stocking density impacted behavior to a lesser extent.

Key Words: Animal Welfare, Broiler Chicken, Environmental Enrichment, Stocking Density, Behavior

M128 Comparison of the Effect of Enrichments on Behaviors of Broilers in Commercial and Experimental Housing Environments Heidi Rinehart^{UG}, Shawna Weimer, Anna Magnaterra, Zoie McMillian *University of Maryland*

Public demands for how chickens are raised has become a topic that the broiler industry is increasingly attuned to provide their consumers. Environmental enrichments increase behavioral opportunities and have the potential to improve broiler welfare; however, the results from scientific research conducted in experimental settings may not translate to practical options on commercial farms. The objective of this study was to compare the behaviors of broilers in experimental pens (Room) to broilers in a commercial house (House) when the birds were in proximity to or interacting with enrichments in their environment. The House was an organic 60 x 600 ft. grow out house with 36,000 broilers and had wooden boxes with hay, ramps, and migration fence enrichments. The Room consisted of six, 5 x 10 ft. pens with about 20 broilers that either had a spotlight, platform, or both spotlight and platform enrichments. Birds were the same age in the House and Room settings. The behaviors of birds either in proximity to or interacting with the enrichments recorded via scan sampling were all preening and aggression, preening, foraging, and dustbathing. Data was analyzed using a one-way ANOVA. Overall, there was a greater proportion of birds interacting with the enrichment in the House (23.6%) than in the Room (17%; $P>0.0001$). In the House, the greatest proportion of birds exhibiting any behavior in proximity to the enrichments was when they were in proximity to the migration fence compared with the other enrichments ($P>0.0001$). The proportion of birds interacting with the enrichment in the Room was the greatest when the birds were in pens with both the platform and spotlight (25.9%), followed by the spotlight (15.9%), and the lowest proportion with the platform enrichment (9.2%; $P>0.0001$). The proportion of birds foraging in the Room was lower (16.1%) for the birds in pens with the platform enrichment compared with birds in pens with the spotlight (15.6%) and both the platform and spotlight (11.4%; $P>0.0001$). Determining differences in the behavior of broilers in experimental and commercial settings will provide the broiler industry with practical information about the effectiveness of environmental enrichments on broiler welfare.

Key Words: Broiler, Behavior, Enrichment, Commercial

M129 Effects of dietary mannan oligosaccharide supplementation on Campylobacter jejuni inoculated broilers Luis Munoz^{GS}, Matthew Bailey, James Krehling, Aidan Talorico, Kaicie Chasteen, Cesar Escobar, Sam Talorico, Maria Elliot, Kenneth Macklin *Auburn University*

Campylobacter infection is the most common cause of human gastroenteritis in the United States. Poultry products are usually associated as the major source of human campylobacteriosis. Therefore, on-farm control of *Campylobacter* in poultry would reduce potential contamination of finished products after processing and would reduce risk of human exposure to this pathogen. A study was conducted to evaluate the effects of diets supplemented with mannan oligosaccharides on *Campylobacter jejuni* colonization in the ceca, growth performance of broilers at days 14, 28 and 41, and carcass *C. jejuni* prevalence after processing. The experiment consisted of a 4 x 2 factorial arrangement of 4 diets (negative control, positive control (bacitracin, 10 g/ton), mannan oligosaccharide constant dose (400 g/ton), and mannan oligosaccharide step-down dose (800, 400 and 200 g/ton in the starter, grower, and finisher periods, respectively). At day 21 two inoculations either *C. jejuni* at 10^7 CFU/ml or 1 ml phosphate buffered saline (PBS) were given. The birds were raised for 44-days (35 birds/pen; 8 replicate pens/treatment); after which, 768 birds were processed. At day 42, all birds, whether inoculated with *C. jejuni* or PBS, had similar levels of *C. jejuni* ($8 \log_{10}$ CFU/ml) recovered from cecal contents and prevalence was similar for all treatments ($P>0.05$). The factor diet did not have an effect in growth performance and the *C. jejuni* inoculated birds had higher body weight and lower feed conversion ratio (FCR) in the finisher period ($P<0.05$). Birds inoculated with PBS and fed with

the positive control diet had higher FCR during the finisher period when compared with those that received mannan oligosaccharides or a negative control diet ($P<0.05$). After processing, the birds inoculated with PBS had higher carcass yield (76.21%) compared to those inoculated with *C. jejuni* (75.09%) ($P<0.05$) and *Campylobacter* prevalence was similar among all treatments ($P>0.05$). These data indicated that mannan oligosaccharides did not reduce *Campylobacter* colonization in the intestinal tract, did not have an impact on growth performance or reduced prevalence after processing. Further studies should evaluate the effect of mannan oligosaccharides over intestinal morphology and immune system regulation.

Key Words: Poultry microbiology, *Campylobacter jejuni*, Colonization, Ceca, Mannan oligosaccharides

M130 The effects of ultraviolet light and darkness on laying hen movement in an aviary housing system Andrea Mendoza^{*1GS}, Shawna Weimer², Zachary Williams¹ ¹Michigan State University, ²University of Maryland

Table egg producers are switching to aviaries as demand for cage-free eggs rises, however the ability to manage birds within the system has proven to be problematic. Therefore, the goal of this experiment was to examine if layer hens will move out of an aviary and onto floor area through a blend of UV lighting and darkening of floor lights. Approximately 1,800 Lohmann Brown hens aged 56 weeks were reared in cage free aviaries (Big Dutchman Natura 60) in four rooms. Each room housed 450 hens in four sections, each room was further divided into three sections with 150 hens per section. Six UV light-emitting diode (LED) blacklight bars were used. Four treatments were as follows: 1) control (C), 2) UV lights turned on for 10 seconds (UV), 3) floor lights turned off, and UV lights were turned on for 10 seconds (UV+DF), and 4) floor lights turned off (DF). Each treatment was applied once in the morning (AM) and once in the afternoon (PM). Video was recorded via closed circuit cameras and hens were observed pre- and post-treatment for changes in spatial distribution and behavior. Six behaviors were recorded: preening (PR), perching (PE), dust bathing (DB), wing flapping (WF), standing alert (SA) and other (O). Data were analyzed in SPSS using GLM at $P<0.05$. Across all treatments for AM session, there was a greater ($P<0.05$) number of hens in the floor area post-treatment application compared to pre-treatment. However, in the PM session, this effect was only seen in the UV+DF treatment. The use of UV lights influenced the behavior of the hens, with more stress related behaviors (PE and SA) observed in the treatments where UV lights were used, while more hens exhibited normal behaviors (PR and WF) in the non-UV light treatments ($P<0.0001$). Although results indicated UV lights moved hens to the floor area of an aviary, this technique may not be effective for long-term movement, but could be used as a control measure for undesirable behaviors that would benefit from immediate short-term movement.

Key Words: Laying hen, Aviary, Behavior, Management, UV light

M131 Comparison of a standard male diet to an organic selenium supplemented male diet on rooster semen quality and reproductive performance Kelly Sweeney^{*GS}, Luis Avila, Jeanna Wilson ^{University of Georgia}

In the US, broiler breeder males are typically fed a female diet out of convenience, but some speculate that a rooster's dietary requirements are not met by a hen formulation. In this study, a basal diet with 2720kcal/kg energy, 12% crude protein, 0.74% calcium, and 0.15ppm of sodium selenite was mixed (Aviagen, 2016) specific to the roosters needs. The basal was split into two diets with the male control (MC) diet containing a total of 0.30ppm of sodium selenite and male organic (MO) diet having 0.15ppm of organic selenium. Selenium is a trace mineral that is important for reproduction and semen quality (Wang et. al, 2017). Organic selenium has a greater bioavailability and retention than inorganic selenium (Li et al., 2018). The objective of this study was focused on evaluating the di-

etary inclusion of organic selenium as a strategy to improve semen production and quality when compared to a traditional male diet. The treatment groups were fed equal amounts of feed from 16 to 65wks. 60 Ross Yield Plus roosters were assigned to each dietary treatment (8 replicates, 10 roosters per replicate). During production, approximately 25 to 30% of males in each treatment were weighed weekly, with groups rotating over time. Percentage of roosters producing semen, semen volume, sperm concentration, and sperm mobility were measured every 5 wks from 25 to 65 wks. Individually caged Ross 708 hens were artificial inseminated with 0.05 mL pooled and diluted semen from a replicate group of males. Semen was diluted with Avian Buffer to 7.5×10^7 sperm. Eggs were collected for 14 days' post-insemination and incubated for a fertility analysis. Data were analyzed by SAS V9.4 using a SLICE analysis and means separated by LSD. Significance level was $P<0.05$. Neither diet had significant influence on body weight, semen volume or 65 wk testicle weight. Sperm mobility was significantly impacted from week 33- 62wks ($P<0.05$) with the males fed the MO diet having greater sperm mobility overall. The organic selenium supplementation had very significant impacts on fertility through 65wks ($P<0.05$) with the males fed the MO diet having greater fertility. Organic selenium has proven to have positive impacts on semen quality and fertility in broiler breeder males during the reproduction cycle.

Key Words: Rooster diet, semen quality, , fertility, organic selenium, reproduction

M132 The role of pullet rearing and hen age on the distribution of layers in multi-tiered aviaries Allison Pullin^{*1GS}, Christina Rufener², Suzanne Millman³, John Tarlton⁴, Michael Toscano⁵, Richard Blatchford¹, Maja Makagon¹ ¹University of California, Davis, ²Center for Proper Housing of Ruminants and Pigs, Federal Food Safety and Veterinary Office FSV, ³Iowa State University, ⁴University of Bristol, ⁵University of Bern

Pullet rearing environments have lasting effects on hen behavior, but their influence on hens' space use in multi-tiered aviaries (MT) is unclear. Our objective was to investigate whether pullet rearing influences the distribution of hens in MT. Dekalb White hens were reared in a floor pen (FL), single-tiered aviary (SI), or two-tiered aviary (TW) until 16 weeks of age (WOA), then moved into MT (30±2 hens/pen, n=5 pens/rearing treatment). Hens were video recorded on two consecutive days at 19, 23, and 27 WOA. At each age, a total of 58 daytime and four nighttime scans identified locations of hens within elevated zones of the MT (A:0.5, B:1.3, C:1.8, D:2.4m). Zones A-C contained a perch and tier. Zone D contained two perches. Effects of treatment and age on the proportion of hens using each zone were analyzed using a linear mixed effects model or a zero-inflated beta regression model. Rearing environment did not affect the total proportion of hens using MT during the day ($P=0.26$) or night ($P=0.35$). There were also no rearing x age interaction effects for day ($P=0.10$) or night ($P=0.41$). However, rearing environment did influence which specific zones the hens used for nighttime roosting through 27 WOA. More FL hens were found in Zone B ($P=0.0002$; estimated mean [95% CI]; FL: 0.21[0.17,0.24], SI: 0.13[0.08,0.18], TW: 0.10[0.08,0.13]) and Zone C ($P=0.003$; FL: 0.74[0.64,0.85], SI: 0.50[0.40,0.61], TW: 0.50[0.40,0.61]), while more SI and TW hens were in Zone D ($P=0.024$; FL: 0.13[0.07,0.23], SI: 0.31[0.22,0.42], TW: 0.30[0.21,0.40]). As they aged, fewer birds were observed utilizing the MT during the day ($P<0.0001$; 19WOA: 0.23[0.22,0.25]; 23WOA: 0.18[0.17,0.20]; 27WOA: 0.15[0.14,0.16]) and more at night ($P<0.0001$; 19WOA: 0.49[0.34,0.64]; 23WOA: 0.56[0.40,0.70]; 27WOA: 0.81[0.70,0.89]). Specifically, all hens increasingly used the highest two zones for nighttime roosting (Zone C: $P<0.0001$; 19WOA: 0.51[0.43,0.60]; 23WOA: 0.55[0.46,0.63]; 27WOA: 0.69[0.61,0.77]; Zone D: $P<0.0001$; 19WOA: 0.16[0.11,0.24]; 23WOA: 0.23[0.18,0.30]; 27WOA: 0.33[0.26,0.41]). Hens acclimate to MT as they age, but FL hens do not fully utilize the highest zone available to them.

These results highlight the role of pullet rearing in acclimation to MT, which may inform management decisions.

Key Words: pullet rearing, aviary, laying hen, behavior

M133 The effect of stocking density stress on commercial white egg layer production parameters Benjamin Alig^{*GS}, Ramon Malheiros, Kenneth Anderson *North Carolina State University*

Stocking density remains a major welfare concern for commercial egg producers, due to the perception by consumers about excess stress on hens. Therefore, it is important to understand how these hens respond to the potential stress proliferated by stocking density. Five different density treatments consisting of 6 birds (208 in²/bird), 9 birds (139 in²/bird), 12 birds (104 in²/bird), 15 birds (83 in²/bird) and 18 birds (69 in²/bird) were examined in colony cage environments. There were six replicates represented by 2 cages of each treatment in colony cages measuring 48 in by 26 in. Data collection for the strains began at 17 weeks of age, with a base period of 4 weeks for feed weigh backs and egg quality measurements and lasted for 12 periods. We hypothesized that hens under higher densities would have worse production metrics than hens with lower densities. Over the whole study density had a significant effect ($p < 0.01$) on Hen Housed production, Hen Day production, and egg weights. We observed that cages with a density of 6 birds had approximately 1.8% higher hen day production than 18 bird cages and 1.4% higher hen day production than 12 bird replicates. Furthermore, the 6 bird replicates were almost 3% higher in hen housed production than the 18 bird replicates. Interestingly, the 18 bird replicates produced heavier eggs by almost 1 gram than the 15 bird replicates. Moreover, this study did not detect any differences in feed consumption and feed efficiency between densities. We also observed significant ($P < 0.01$) differences in % USDA Grade A eggs and % USDA Loss eggs however, we did not observe any differences in USDA egg size percentages between densities. 6 bird replicates laid approximately 4% less grade A eggs and 4% more loss eggs than 12, 15 and 18 bird cages. In conclusion, birds in high density cages exhibited expected egg production characteristics however, in contrast to expectations, higher density cages had better USDA egg grades, heavier eggs and similar feed consumption, feed efficiency and USDA egg sizes to hens in lower density cages.

Key Words: Laying Hens, Stress, Stocking density, Production, USDA Grades

M134 An assessment of *Heterakis gallinarum* transmission between poultry farms Justin Lowery^{*UG}, Catherine Fudge¹, Brian Wooming², Katherine Cupo¹, Olivia Wedegaertner¹, Lin Walker¹, Chongxiao (Sean) Chen¹ *¹North Carolina State University, ²Prestage Department of Poultry Science, ²Cargill, Inc.*

Heterakis gallinarum, a cecal worm, is the only known vector for the protozoan parasite *Histomonas meleagridis*, which causes histomoniasis. Understanding the environmental population and epidemiology of *H. gallinarum* is important to assess histomoniasis outbreak risk in turkey farms. *H. gallinarum* cycles in chickens but seldom in turkeys since it frequently carries *H. meleagridis*, resulting in quick death before *H. gallinarum* egg production. The only known vectors for *H. gallinarum* are earthworms, fomites, and potentially darkling beetles. We conducted two studies examining epidemiological factors of *H. gallinarum* in poultry farms and their environments. First, the relationship of cecal worm load in organic laying hens with *H. gallinarum* eggs in litter was investigated to attempt to estimate environmental *Heterakis* loads in chicken houses. Five birds per farm from fifteen organic laying hen farms (75 birds), were collected for cecal *H. gallinarum* counting. Litter samples were also collected from the same farms (four sampling points in each house where the birds were raised) for *H. gallinarum* egg counting. The data was analyzed using Pearson Correlation Coefficient analysis to examine the relationship between cecal worm population and litter egg counts. The results showed that all farms housed birds infested with *H. gallinarum* and 53.33% of the farms con-

tained eggs in their litter. However, no correlation existed between cecal worm load and *Heterakis* egg abundance in the environment ($P = 0.5374$, $R^2 = 0.0299$). Our second study focused on screening potential vectors in or near turkey houses with histomoniasis outbreaks using necropsy and a *H. gallinarum* PCR test. Beetles, flies, insect larvae, grasshoppers, crickets, and earthworm were tested, but only darkling beetles and earthworms returned positive results. In summary, there is no correlation between cecal worm load and *Heterakis* egg abundance in the environment from this study, indicating that chicken *H. gallinarum* infestation may not represent environmental egg load. Darkling beetles and earthworms may carry *Heterakis* between farms, suggesting insecticides and strategic location planning may help prevent the introduction of insect vectors to turkey farms and reduce the possibility of a histomoniasis outbreak.

Key Words: *Heterakis gallinarum*, Histomoniasis, *Histomonas meleagridis*, Environment, Vector

M135 Continuous environmental noise pollution and gizzard koilin layer erosion in young turkey poult Chloe O'Brien^{*UG}, Olivia Wedegaertner, Catherine Fudge, Katherine Cupo, Frank Edens, Chongxiao (Sean) Chen *North Carolina State University, Department of Poultry Science*

In current animal husbandry, noise pollution has been seldom investigated. Acute and chronic stressors from noise pollution can cause birds to behave abnormally due to fear responses. Such responses could reduce water and feed consumption predisposing poultry to enteric disease. Here we report a case on a research farm associated with high mortality, high morbidity, and severe necrosis and sloughing of the gizzard koilin lining in young turkeys from one of two research rooms. Turkey poults from the same hatchery were placed into 2 battery cage rooms involving 2 separate trials. 400 birds were placed in room 1 (R1) and divided among 40 cages and 720 birds were divided among 72 cages in room 2 (R2). Before the trials began, it was noted that the ceiling circulation fan in R2 was emitting a loud sound, much louder than the fan in R1, and was replaced on D15. For the first two weeks, birds in R2's were more lethargic, seen eating less, and huddled in the back of their respective pens compared to birds in R1. R2 had a high mortality rate (16.5%) compared to R1 (9.75%) at d18. Furthermore, the mortality of each cage is higher when getting closer to the ceiling fan in R2. The average noise level was 91.4 dBA and 3297Hz in R2 vs. 90.1dBA, and 4736Hz in R1. After replacing the fan, birds in R2 resumed normal eating and drinking behavior but had significantly reduced growth, compared to R1 birds (-0.1184 kg/bird on average at D21 [$p < .0001$]). On days 14 and 29, birds from both battery cage rooms were taken to a diagnostic laboratory for necropsy. Severe white necrosis and sloughing of the gizzard koilin lining was reported and results were positive for *Clostridium* in the gizzard. Evidence supports that the consistent environmental noise stressor caused birds in R3 to become severely depressed and lethargic, perhaps suffering from an avian form of stress-induced anorexia. A lack of feed consumption likely caused an overproduction of gastric acid inside the gizzard leading to hydrolysis of the koilin layer. A compromised lining of the gizzard could have allowed an opportunistic bacterium, such as *Clostridium*, to express itself. This case report suggests that continuous noise pollution can adversely affect the productive performance and behavior of birds.

Key Words: environment, noise pollution, stress, gizzard erosion, behavior

M136 Role of environmental noise on livability and behavior in young turkey poults Junjie Yang^{*UG}, Catherine Fudge, Olivia Wedegaertner, Katherine Cupo, Frank Edens, Chongxiao (Sean) Chen *North Carolina State University*

Intermittent environmental noise leads to changes in poultry behavior; however, few studies have been done to observe the effects of continuous environmental noise. two studies were conducted to investigate the role

of environmental noise on livability and behavior in turkeys. In study I, data was obtained from a turkey research trial: 720-day-old poults were placed into 72 battery cages. A stirring fan was placed on the ceiling in the middle of the room and left on. A bearing was broken in the fan motor, causing increased loudness and an oscillating frequency. Poults were raised for 4 weeks and monitored daily for morbidity and mortality. Noise levels were collected in each pen using a sound meter (Go Direct® Sound Sensor) for A-weighted, C-weighted sound level, and frequency. Mortality from each was correlated with noise level using Pearson Correlation Coefficient analysis (SAS 9.4, $P < 0.05$). For study II, 50 poults were allocated to two separate rooms. 30 poults (3 cages x 10 birds) were placed in room 1 with similar environmental conditions as study I (utilizing the broken fan). 20 poults were placed in room 2 under standard environmental conditions for turkeys. A Go-Pro was placed in front of the cages

in each room and set to collect a photo every minute. For four days images were collected of poults. Bird behaviors between rooms 1 and 2 were recorded and compared using Student t test (SAS 9.4, $P < 0.05$). Study I showed no correlation between the sound level, frequency, and the mortality in each cage, which may be attributed to the similarity in sound levels between cages. In study II, the birds in room 2 showed an increase in sitting behavior ($P = 0.0228$) and a decrease in standing behavior compared to room 1 ($P = 0.0340$). It is arduous to determine a definitive sequence of events for this case report but much of the evidence fortifies a consistent environmental noise causing the birds in room 1 to become astringently dejected and lethargic. Noise as an environmental factor in domestic fowls has been little investigated; this study suggests that environmental noise can adversely affect the livability and behavior of the birds.

Key Words: Noise, mortality, turkey

Environment, Management and Animal Well-Being: Environmental Impacts

M137 Achieving production gains by preventing pathogen proliferation and biofilm formation in the gastrointestinal tract of poultry Michael Barnas*, James McNaughton, Mick Roberts *AHPharma, Inc.*

Chickens consume approximately twice the amount of water as feed. Consequently, administering water-soluble ingredients through the drinking water lines delivers substantial amounts of supplemental nutrients to the flock. Dosing specific supplements in the drinking water for abbreviated periods, like 8 hours daily, has achieved similar or improved efficacy at a lower cost than continuous administration. The first goal of this research project was to engineer a water-dosing system that allows poultry growers to automate the administration of supplements alone or in combination with other ingredients to achieve significant reductions in water-line bacteria loads during house layout periods, just before flock placement, and during the grow-out. The water medication system was designed with an automatic flushing function that ensures the water lines remain pressurized and primed with the water supplements at all times. This function can also systematically eliminate biofilm by completely emptying water lines, then re-filling them with disinfection chemistry, and finally flushing with fresh water. Experiments were conducted to evaluate administering various water supplements, including antimicrobials and probiotics, to broiler chickens using the medication system. *Salmonella* Enteritidis, Typhimurium, and Heidelberg were used in the experiments, and 5 mg/L chlorine dioxide (ClO_2) demonstrated antimicrobial activity for each serotype compared to control. Oxidants like chlorine dioxide react directly with the cell wall of microorganisms and theoretically can destroy any pathogens of concern like *Salmonella* and *Campylobacter*. In this study, dosing ClO_2 at 5 mg/L of drinking water for 8 hours daily reduced *Salmonella* to 0% incidence in the small intestine contents, cecal contents, and homogenized gut tissue of 42d broilers previously challenged via oral gavage with 10^4 CFU *Salmonella* per bird at 7 and 40-42 days of age. Practical use of this dosing strategy could improve water quality by reducing biofilm, accentuating the efficacy of other feed and water, significantly reducing fecal pathogens before processing, and preventing further pathogenic cross-contamination during the initial stages of meat processing.

Key Words: Dosing, Chlorine dioxide, *Campylobacter*, *Salmonella*, Pathogen

M138 Determination of bacterial pathogen diversity and prevalence in acidified re-used poultry litter during commercial live production Adelumola Oladeinde*, Michael Rothrock, Charlene Jackson, Mark Berrang, Maggie Weinroth, Anthony Pokoo-Aikins, Anthony Glenn, Scott Gold *U.S. National Poultry Research Center; USDA-ARS*

Acid amendments are used for treating re-used poultry litter between broiler chicken flocks for in-house ammonia abatement. These amend-

ments reduce litter pH and inhibit NH_3 volatilization by converting NH_3 into nonvolatile NH_4^+ , however their effect on bacterial abundance and the prevalence of pathogens is not fully understood. In this study, we investigated the diversity and changes in abundances of several bacterial foodborne pathogens (*Salmonella*, *Campylobacter*), commensal bacteria (*Staphylococcus*, *E. coli*, *Enterococcus*) and fungi in re-used litter from a commercial poultry house during grow-out, downtime (period after a flock was removed and before new broiler chicks were placed) and after liquid aluminum sulfate application. Our findings from three flocks show a reduction in *Salmonella* prevalence and *Staphylococcus*, *E. coli* and *Enterococcus* population abundance during downtime and after aluminum sulfate application. However, these bacterial populations either returned to pre-downtime/acidification levels or exceeded them towards the end of the grow-out period. *Campylobacter* was detected in all litter samples ($n = 16$) at the end of the 1st flock but was not detected in litter samples collected from successive flocks ($n = 48$). Likewise, there was a reduction in litter pH and moisture during downtime ($\text{pH} = 7.14 \pm 0.016$, moisture = $23.3 \pm 0.013\%$) and after aluminum sulphate application ($\text{pH} = 4.90 \pm 0.15$, moisture = $19.5 \pm 0.0021\%$), but these litter physio-chemical parameters increased ~ two weeks after chick placement ($\text{pH} = 6.59 \pm 0.030$; moisture = $25.9 \pm 0.013\%$) and by the end of the grow-out (6.82 ± 0.042 ; moisture = $44.3 \pm 0.016\%$). These results suggest that downtime and litter acidification have a different effect on *Salmonella* persistence in pre-harvest compared to *Campylobacter*; and further studies are needed to disentangle this relationship.

Key Words: Poultry litter, Acid amendment, Downtime, Pathogens, pH and moisture

M139 Evaluating *Salmonella* population dynamics in broiler flocks Tomi Obe*, Nikki Shariat *University of Georgia*

Consumer concerns have led integrators to reduce their use of antibiotics and most broiler flocks are now raised without antibiotics of human medical importance (NAHMI) or no antibiotic ever (NAE) flocks. Poultry is a major reservoir for *Salmonella*, a leading bacterial cause of foodborne illness in the United States. *Salmonella* serovars are extremely diverse in their response to mitigation strategies and ability to cause illness. Understanding *Salmonella* serovar dynamics during live production is vital to reducing overall levels on broilers, leading to improved mitigation at processing. We evaluated *Salmonella* on 20 broiler farms (80 different flocks) and compared *Salmonella* incidence, quantity, and serovar populations between broilers raised under NAHMI and NAE production systems. The flock ages ranged from three to five weeks and all samples were collected within a five-week period to limit seasonal effects. Two pairs of boot-socks were used in each flock (one flock per house) to collect samples,

and *Salmonella* was isolated following standard enrichment protocols. Positive samples were quantified using Sal-Quant (Hygiena) and CRIS-PR-SeroSeq was used to evaluate serovar populations within each broiler flock. Results were analyzed using completely randomized ANOVA with Tukey's HSD means separation ($p \leq 0.05$). *Salmonella* incidence was not different ($p=0.55$) between the two production systems (NAHMI = 82.5%, NAE = 87.5%), however a slight difference was observed between farms ($p=0.06$). *Salmonella* quantity also did not differ between the production systems ($p=0.15$) and farms ($p=0.21$) with a mean Log CFU for NAHMI = 3.40 and NAE = 3.90. Serovars Kentucky, Typhimurium, Schwarzengrund, Enteritidis, and Infantis were the most detected serovars across both production systems. In terms of *Salmonella* population complexity, we found up to five serovars in a single flock, with an average of two serovars per flock. There was no difference in serovar complexity between flocks. Though a small study, these initial data suggest that production systems do not impact *Salmonella* incidence, quantity, or complexity of serovar populations. This information can help integrators develop targeted controls towards persistent *Salmonella* serovars in their complexes.

Key Words: Salmonella, Broiler flocks, NAE, Antibiotics, Processing

M140 Impact of environmental enrichment on performance of commercial broiler Muhammad Shahid Zahoor¹, Sohail Ahmad¹, Muhammad Dawood², Murrawat Hussain¹, Muhammad Adeel Maqsood^{1,3}, Hafiz Rao Abdul Latif¹ ¹Department of Poultry Production, University of Veterinary and Animal Sciences, ²Department of Animal Breeding and Genetics, University of Veterinary and Animal Sciences, ³Department of Animal Nutrition, University of Veterinary and Animal Sciences

This study evaluated the effect of environmental enrichment on the performance of broiler chickens. A total of 360 straight run broiler chicks (Ross-308) were divided into 6 treatment groups having 4 replicates of 15 birds each under completely randomized design. Treatments were environmental enrichment (EE) tools and consisted of C = Control group; R = Red ball for EE; G = Green ball for EE; B = Blue ball for EE; M = Mirror for EE, and L = LED on feeders. These environmental enrichment tools were provided throughout the experimental period (0 to 35 days) and their effect was evaluated on growth performance, behaviour and welfare aspects, blood biochemistry, carcass, and meat quality traits. Mean feed intake per bird was higher in birds having a light on feed as environmental enrichment; the highest weight gain and feed conversion ratio was better in the green balls group. Broiler chickens reared under different environmental enrichment were more active and they exhibited maintenance behaviour (preening, dust bathing, and wing stretching, or scratching) more frequently. Regarding welfare, the green and blue balls group had a lower incidence of toe damage and hock burn. The birds reared with red balls as environmental enrichment showed the lowest values for glucose, cholesterol, total protein, albumin, and globulin among all the treatment groups. Birds reared with green balls had the highest body weight at slaughter, dressed weight, carcass yield, and liver weight. Breast meat of environmentally enriched treated groups was lighter and had lower ultimate pH. It was concluded that the addition of environmental enrichment tools (visual, structural, and plastic) motivates the birds for physical activities and improves the performance of broiler chickens.

Key Words: Environmental enrichment, growth, welfare, meat quality traits, broiler chickens

M141 Light stimulation during incubation can influence hatching traits and post-hatch performance of broilers Jibran Hussain^{*}, Muhammad Faisal Riaz *Department of Poultry Production, University of Veterinary and Animal Sciences*

Incubation is one of the most important steps for better development and growth of chicken, as the environment during embryogenesis has lifetime effects on the performance and well-being of birds. Just like temperature, humidity, turning and ventilation, light also has very important role in em-

bryonic growth and providing light during incubation stage of broilers can help the birds to adjust in the pre and post-natal environment as embryo start to respond to light from the second day of incubation. Yet, this factor not studied as much comprehensively as it should have been. Hence, keeping in view such scenario a comprehensive study was conducted at the Department of Poultry Production, UVAS Lahore, Pakistan. In this trial a total of 900 broiler breeder eggs of the same weight from the same flock were exposed to different intervals of photo-stimulation including zero (0), twelve (12) and twenty four (24) hours through using LED Bulbs to study the hatching traits in terms of egg moisture loss during incubation, early, mid and late embryonic mortality patterns, hatch of fertile, hatch window and overall hatchability. Stress response in terms of heterophil/lymphocyte ratio, physical asymmetry, feather score and gait score was also studied in response to light stimulation. Further, post-hatch broiler performance in terms of feed intake, body weight gain and FCR was also examined. Improved hatch window, overall hatchability and chick quality were observed in response to light stimulation for 12 hours. Likewise, a significant improvement was observed in stress response as well as growth performance of broilers in response to light stimulation, especially for 12 hours. Considering the appreciable results of the present study, it is recommended to adopt 12 hours of light stimulation during incubation as a prerequisite factor to attain improved hatching traits, stress response and overall growth performance of broilers.

Key Words: Light stimulation, hatching traits, stress response, growth performance

M143 Evaluation of betaine hydrochloride on Lohman Brown layers 21-77 weeks of age; A Field Study Kim Wilson¹, Regina Harris², Michael Persia³, Lien Vande Maele¹ ¹*Orffa Additives, B.V.*, ²*Heritage Poultry Management Services*, ³*Virginia Tech*

Betaine is a compound well established for its function as a protective osmolyte in cells. Betaine can replace added choline as a methyl donor. Any extra available betaine molecules are able to infiltrate cells to maintain integrity and electrolyte homeostasis, thus by hypothesis enable cellular efficiency and influence animal production. However, peer-reviewed betaine studies are often with concentrations that are not practical in commercial diets. Therefore, the objective was to examine effects of added choline replacement by betaine at equimolar equivalence in commercial layers. The design was a paired house trial with cage-free Lohmann brown layers, placed at 16.5 weeks on the same diet. The trial began at 21 weeks and ended at 77 weeks with the following treatments: CON: basal diet with choline chloride 60% at 500 ppm; BET: CON diet replacing choline chloride with 348 ppm betaine HCl, with bird numbers at 20,018 and 19,991, respectively. At 35 and 45 weeks, litter moisture and egg quality parameters from 80 fresh eggs were measured. At 55, 65 and 75 weeks,

half of the eggs were measured immediately (FRESH) while the other half remained in room temp for 7 days before measurements (7-DAY). Chi square was performed to detect differences in total mortality. An unpaired t-test was performed to analyze litter moisture and egg quality data were subjected to a two-way ANOVA factoring treatment and time. Cumulative mortality, although high due to disease pressure in both houses, was reduced ($P < 0.05$) in BET (22.3% vs 18.6%). Litter moisture was reduced in BET during winter months, 35 and 45 weeks (CON $21.9\% \pm 0.10$; $19.5\% \pm 0.11$; BET $18.1\% \pm 0.25$; $16.3\% \pm 0.18$). Beginning at 55 weeks, there was a time ($P = 0.03$) and treatment effect ($P = 0.04$) on 7-DAY eggs where BET increased albumin height (AH) and Haugh units (HU) (AH CON $3.7\text{mm} \pm 0.13$ BET $4.2\text{mm} \pm 0.24$; HU CON 52.4 ± 1.82 BET 57.8 ± 2.54). Both BET FRESH and BET 7-DAY had improved AH and HU relative to respective CON groups. By 75 weeks, numerical improvement was observed BET FRESH (AH CON 4.9 ± 0.19 BET 5.3 ± 0.62 , $P = 0.20$). Taken together, betaine can replace added choline and extra betaine can support layer production by reducing mortality and litter moisture while improving egg quality and freshness.

Key Words: layers, betaine, litter moisture, egg production, choline

Physiology, Endocrinology and Reproduction: Layer or Broiler Breeders

T145 Effect of breeder age, egg storage length, and SPIDES on hatchability and embryo mortality of brown and leghorn layer breeders Gustavo Quintana-Ospina¹, Maria Alfaro-Wisaquillo¹, Edgar Oviedo-Rondón¹, Ian Rubinoff², Daniel Valbuena² ¹*North Carolina State University*, ²*HyLine International*

Incubation parameters of layer breeders are not well known. A data analytics study from commercial hatcheries was conducted to evaluate the fertility, hatchability, hatch of fertile (HOF), and embryo mortality (EM) parameters of brown (BB) and leghorn (LB) breeds. Two datasets containing hatchability and embryodiagnosis records were collected from 545 layer breeder flocks between 2014 and 2020 in 13 hatchery plants. One dataset included 45,626 hatches representing 919 million white leghorn and 195 million brown hatchlings. The second dataset reported 14,256 breakouts that involved early, mid, and late EM, contaminated and cracked eggs. Both databases included flock age, egg storage time, and whether a SPIDES treatment was applied or not. Egg storage time was grouped into 5-day periods. Data were analyzed using a one-way ANOVA from 23 to 70 wk of age with the breed, egg storage time, and SPIDES treatment as main effects and hatchery as a random effect. The LB had on average 3.80% higher ($P < 0.05$) hatchability (81.84 vs. 78.04%) and 2.60% more HOF (84.59 vs. 81.98%) than the BB flocks. In both breeds, the best hatchability was reached at 35 wk with a gradual decrease afterward. The LB flocks were more fertile at all weeks evaluated ($P < 0.05$), except for wk 61, 66, 67, and 69. The average difference in fertility was 0.86% between breeds (97.90 vs. 97.04%). Early EM was split into membranes, EM with less than two days, and blood ring stage deaths. In the early membrane, differences were observed from 24 to 41 wk and intermittently between 44 and 70 wk. An average of 0.65% more dead embryos in this phase were observed in BB. In contrast, significant differences ($P < 0.05$) on the blood ring stage were detected only at 30 and 60 wk. Mid-EM was less than 1.3% in all periods, and LB accounted for higher mortality rates ($P < 0.05$) at 27, 40, 45, 46, 56, and 63 wk. However, the difference between breeds on mid-EM was observed to be, on average, 0.27%. Breeds also differed ($P < 0.05$) on late EM at multiple ages. Brown embryos presented late mortality rates (3.14%) ranging from 0.05 to 0.99% higher ($P < 0.05$) than their leghorn counterparts (2.18%) across the production cycle. Data analytics helped to determine average incubation parameters for leghorn and brown layer breeders.

Key Words: Data analytics, Incubation, Layer breeders, SPIDES, hatchability

Physiology, Endocrinology and Reproduction: Turkeys

T146 Using the i-STAT and the VetScan VS2 analyzers to formulate age-specific whole blood biochemistry and gas reference intervals in brooder production turkeys Zachary Adams, Rocio Crespo* *Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University*

Modern point of care blood analyzers, such as the i-STAT and the VetScan VS2 Analyzers, are commonplace in all aspects of the veterinary field; however, these analyzers are rarely used by the poultry industry. This is a lost opportunity for the industry as a whole, as the implications that the use of these analyzers can have on the poultry health industry are significant. The use of these analyzers can lead to early detection of disease processes, environmental stressors and can help with regular flock health monitoring. To establish the reference intervals, we tested healthy meat type turkeys

from 6 independent flocks during a 3-year period. Blood samples were then divided in brooder (<1 week) and growing (1-12 weeks of age). A total of 330 blood samples were tested with the i-STAT and 234 by the VS2. Significant differences ($p < 0.05$) in the means between the two age groups (brooder and growing) were determined using Mann-Whitney. Reference intervals were calculated by a non-parametric method using the Reference Value Advisor v2.1. Additionally, box and whiskers plots were used to visualize daily changes in blood analytes during the first week. This study provides the industry with an opportunity to start interpreting blood analysis results in production turkeys, during the brooding and growing stages of their lifecycle. It offers veterinarians a new approach to monitoring and identifying metabolic health concerns, which are vital for poult success.

Key Words: Turkey, Parameters, Poult

Metabolism and Nutrition: Vitamins and Minerals

T147 Effects of the *in ovo* administration of L-ascorbic acid on the growth performance and incidence of eye lesions in broilers subjected to elevated levels of atmospheric ammonia Ayoub Mousstaaid*¹, Saman Fatemi¹, Katie Elliott¹, Edgar Peebles¹, Joseph Purswell², William Miller³, Hammed Olanrewaju² *¹Mississippi State University, ²United States Department of Agriculture - ARS, ³Animal Ophthalmology Clinic*

The live performance and physiological variables of broilers in response to the *in ovo* injection of L-ascorbic acid (L-AA) under normal conditions has been previously investigated. However, the effects of *in ovo* injection of L-AA on the performance of broilers challenged with elevated atmospheric ammonia levels throughout the rearing period have not been previously reported. Therefore, the objective of the current study was to determine the effects of the *in ovo* injection of various levels of L-AA on the performance and incidence of eye lesions in Ross 708 broilers exposed to 50 ppm of atmospheric ammonia after hatch. A total of 1,440 Ross 708 broiler embryos were randomly assigned to 4 treatments: non-injected (control), sterile 0.85% saline-injected (control), or saline containing 12 or 25 mg of L-AA. An Inovoject multi-egg injector machine was used at 17 d of incubation to administer a 100 µL volume of each prespecified treatment. At hatch, 12 male chicks were randomly assigned to each of 48 battery cages with 12 replicate cages randomly assigned to each treatment group. All birds were exposed to 50 ppm of ammonia for 35 d and the concentration of ammonia in the battery cage house was recorded every 20 sec. Mean BW, BW gain (BWG), average daily BW gain (ADG), and feed intake, as well as feed conversion ratio (FCR) were determined weekly. From 0 to 35 d of age (doa), 6 birds from each cage were selected and sampled for eye lesion scoring. A randomized complete block experimental design was used. All live performance data were analyzed using one-way ANOVA and a repeated measures analysis was used to analyze lesion score data. No significant differences were observed among treatments for incidence of eye lesions. The *in ovo* injection of 12 mg of L-AA increased BWG ($P=0.043$) and ADG ($P=0.041$), and decreased FCR ($P=0.043$) from 0 to 28 doa in comparison to saline-injected controls. The posthatch improvement in broiler performance in response to the *in ovo* injection of 12 mg of L-AA may be associated with an increase in the antioxidant capacity of the birds. Further study is needed to determine the effects of *in ovo* injection and dietary supplementation of L-AA on the antioxidant activity of broilers during the exposure of elevated atmospheric levels of ammonia.

Key Words: Ammonia, Eye lesion, Growth performance, *In ovo* injection, L-ascorbic acid

T149 Performance and immune parameters of broilers under Eimeria challenge and fed copper (I) oxide without antibiotic Naiana Einhardt Mancke*¹, Alessandra Monteiro¹, Cristiane Araujo², Cristina Massoco Salles Gomes², Fabricia de Roque Arruda², Lucio Araujo² *¹Animine, ²University of Sao Paulo*

The objective of this study was to evaluate the effect of copper (I) oxide (Cu_2O , CoRouge®, Animine, France) on performance and immune parameters of broilers under Eimeria challenge. A total of 600 one-day-old male broilers (Cobb 500) were allocated to one of four dietary treatments according to a randomized complete block design. Each treatment had 10 replicates pens and 15 broilers per pen. The treatments were as follow: Negative control (NC) - without antibiotics (AntB); Positive control (PC) -50 mg/kg of zinc bacitracin; NC + 150 ppm of Cu from Cu_2O ; PC + 150 ppm of Cu from Cu_2O . At day 7, broilers were given 12x the manufacturer recommended dose of Bio-Coccivet R® (Biovet) by oral gavage. Broilers and feed were weighed by pen at 0, 14, 28, and 42 d. Mortality and

post-mortem weight were recorded daily for the calculation body weight gain, and FCR corrected by mortality. On day 21, blood was collected from one bird per pen to assess phagocytosis rate and oxidative burst. Data was analysed by ANOVA using the GLM procedure of SAS. Differences among means were tested by using Tukey's test. Broilers fed NC diets presented the worse ($P>0.05$) growth performance and phagocytosis rate compared to other treatments. The combination of AntB+Cu presented the highest BW ($P<0.001$) compared to AntB alone, while Cu treatment had intermediate BW value. Feed intake follow the same trend on days 21 and 35, while no statistically difference was observed ($P>0.05$) on the overall period. On day 21, AntB+Cu presented better FCR ($P=0.006$) compared to AntB alone, however did not differ from broilers fed only Cu. On day 35, the broilers fed Cu diets improved FCR ($P<0.001$) compared to other treatments, whereas the combination of AntB+Cu was statistically better than AntB alone. Overall, broilers fed AntB+Cu and Cu alone had better FCR ($P<0.001$) compared to broilers fed only AntB. Regarding phagocytosis rate, broilers fed diets with Cu and without AntB ($P<0.001$) had higher levels compared to AntB+Cu and AntB alone, while AntB alone had lower levels than the combination of AntB+Cu. In conclusion, the use of 150 ppm of Cu from Cu_2O may promote better performance and higher phagocytosis rate of broilers under *Eimeria* challenge compared to AntB.

Key Words: Trace-minerals, Poultry, Antimicrobials

T150 Effects of the *in ovo* administration of the Marek's Disease Vaccine alone or in combination with the *in ovo* and supplemental dietary administration of 25-hydroxyvitamin D₃ on the performance and meat yield of Ross 708 broilers Saman Fatemi^{*1}, Katie Elliott², April Levy³, Edgar Peebles¹ ¹Mississippi State University, ²USDA-ARS, ³DSM Nutritional Products

The separate administration of dietary or *in ovo*-injected 25-hydroxyvitamin D₃ (25OHD₃) has been shown to improve broiler performance and meat yield. However, the possible synergistic effects of both dietary and *in ovo* of 25OHD₃ sources in conjunction with the Marek's Disease Vaccine (MDV) have not been previously reported. The objectives of this study were to determine effects of the MDV alone or in combination with the *in ovo* and dietary administration of 25OHD₃ on the live performance and breast meat yield of Ross 708 broilers. Live embryonated hatching eggs were randomly assigned to one of the following 4 *in ovo* injection treatments at 18 d of incubation. 1) Non-injected; 2) MDV alone; or MDV containing either 3) 1.2, or 4) 2.4 µg of 25OHD₃. A 50 µL solution volume was injected into each egg using an Inovoject multi-egg injector. Birds were assigned to the one of the following posthatch dietary treatments: 1) commercial diet containing 250 IU of vitamin D₃ /kg of feed (control); or 2) commercial diet supplemented with an additional 2,760 IU of 25OHD₃ /kg of feed (HyD- diet). In the grow out period, each treatment was replicated in 6 pens (48 total pens), each containing 14 chicks. The BW, BW gain (BWG), feed intake, and feed conversion ratio of the birds were determined in each dietary phase. At 14 and 40 doa, one bird within each of the 6 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. A randomized complete block experimental design was used and all data were analyzed using a two-way ANOVA. No significant dietary x *in ovo* treatment interactions were observed for any of the variables examined. The BW, BWG, FI, and FCR from 0 to 40 ($P<0.0001$) doa and breast meat yield ($P<0.0001$) at 14 and 40 doa were improved in response to dietary 25OHD₃ in comparison to the commercial diet. An improvement in breast meat yield and live performance in the broilers belonging to the dietary 25OHD₃ treatment may be due to an improvement in immunity or small intestine morphology which may result in increased nutrient absorption. However, future research is needed to determine the aforementioned effects.

Key Words: 25-hydroxyvitamin D₃, Breast meat yield, Broilers, *in ovo* administration, Marek's Disease Vaccine

T156 Evaluation of the effects of different 25-Hydroxyvitamin D3 sources in broiler chickens subject to a necrotic enteritis challenge April Levy, Chasity Pender, Shelby Corray^{*} *DSM Nutritional Products*

Dietary inclusion of 25-Hydroxycholecalciferol (25-OH-D3) and Vi-tamin D3 has demonstrated benefits in skeletal health, muscle growth, and immune health for broilers. The objective of this experiment was to evaluate two 25-OH-D3 sources (A and B) on performance and intestinal immune-related gene expression of broiler chickens undergoing a *Clostridium perfringens* (CP) challenge. A total of 400 day-old Ross male chicks were randomly allocated to one of four treatment groups, T1: a non-supplemented control; T2: product A[PC1] (25-OH-D3; HyD[®]) di-etary supplementation at 69 µg/kg during the starter phase (d0-14), T3: product A dietary supplementation at 69 µg/kg in the starter (d0-14) and water application of product A at 1 oz/128 gal during the grower phase (d14-28); and T4: product B (VitD3/25-Hydroxyvitamin D3 mix product) at 34.8 µg/kg in the starter (d0-14) and water application of product A at 1 oz/128 gal during the grower phase (d14-28). Each group consisted of 10 replicate battery cages with 10 birds per cage. On d14, all groups were challenged via oral gavage with *E. maxima* (5,000/bird) followed by oral CP inoculation (1x10⁸ CFU/bird) on d19, 20, and 21. Prior to the challenge, no significant differences were noted in BW, FI, or FCR. On d22, at the peak of the challenge, T3 and T4, which were being supplemented with product A in the water, had the lowest non-adjusted FCR; however,

these differences were not significant. T1 had a significantly higher necrotic enteritis (NE) related mortality (47%) compared to T2 and T4 (33%; $P < 0.05$), while T3 was intermediate (35%). On days 19, 20, 21, 22, and 23 fecal samples were collected from each cage. The only coccidia identified was *E. maxima* and there were no significant differences in oocyst numbers between the groups at any of the sampled timepoints; however, T2, T3, and T4 had numerical reductions in coccidia OPGs versus T1. On d21, expression of IL-1 β in the ileum was highest in T3 (3.73) compared with T2 and T4 (1.12, 1.07, respectively). On d22 expression of IL-17A in the ileum was higher for T2, T3, and T4 (14.08, 14.00, 13.68, respectively) compared with T1 (11.70). Overall, water supplementation of product A (25-OH-D3; HyD[®]) improved the performance outcomes and positively influenced immune markers.

Key Words: 25-Hydroxyvitamin D3, coccidiosis, broiler, cytokines

SCAD

T157 Comparison of ATP Bioluminescence to Traditional Colony Counting for Bacterial Enumeration of Treated and Non-treated Hatching Eggs

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Bacterial contamination of hatching eggs can have significant economic impacts to the broiler industry due to decreased hatchability from embryo mortality and increased chick mortality in the first week of life. Traditionally, bacterial quantification of hatching eggs has relied on bacterial culture and colony counting, which is time consuming and labor intensive. Rapid adenosine triphosphate (ATP) bioluminescence assays have become widely used in the food and healthcare sectors to monitor surface cleanliness by quantifying ATP levels, providing an indirect measurement of microbial load and organic contamination. In this study, we compared the results of ATP bioluminescence and bacterial colony counting on hatching eggs treated with two different disinfectants. 180 commercial broiler breeder hatching eggs were randomly divided into three treatment groups: hydrogen peroxide treated (60), quaternary ammonium treated (60), and untreated controls (60). Eggs were sampled prior to application of products, immediately after application, and 24, 48, 72 and 96 hours post application. Individual eggs were sampled once over the course of the study, using both methodologies. The results of the ATP bioluminescence assay and bacterial colony counting were found to be significantly positively correlated ($p < 0.0001$). This finding suggests ATP bioluminescence assays may be a reliable method for assessing bacterial contamination of surfaces. These assays have the advantage over traditional colony counting methods of being a user-friendly option, providing real-time results, and reducing the time and resources required for processing samples. A preliminary field study was also performed to evaluate the effects of the quaternary ammonium product on commercial hatching eggs through incubation and the first week of life. The product was applied by electrostatic spray to 42,240 eggs on the breeder farm prior to transport to the hatchery. An equal number of untreated eggs were tagged as the negative control group. 33,696 chicks hatched from treated and untreated eggs were placed on the same farm in adjacent houses. Results of residue breakouts of unhatched eggs, necropsy with bacterial and fungal culture of one-day-old chicks and 7-day mortality and body weights will be presented.

Key Words: ATP Bioluminescence Assay, Bacterial quantification, Hatching eggs, Disinfection

T158 Antibody response to *Salmonella* Enteritidis siderophore receptor and porin (SRP®) protein vaccines using a proprietary ELISA

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Salmonella Enteritidis (SE) control in poultry requires a comprehensive program that starts during live production and continues through processing and marketing of poultry and egg products. Integral to the program is the vaccination of breeder and layer flocks to induce protective antibodies prior to lay. An innovative technology using immunogenic siderophore receptor and porin (SRP®) proteins present in all *Salmonella* serotypes adds to the arsenal of vaccines against SE.

Antibodies specific to SRP proteins are generated upon administration of these new SRP technology vaccines. However, commercial *Salmonella* ELISA kits do not detect these SRP-specific antibodies very well. A proprietary SRP-specific ELISA was therefore developed to detect SRP-specific antibodies.

A series of studies was conducted to demonstrate the immune response and production of SRP-specific antibodies after vaccination with *Salmonella* SRP vaccines. We wanted to determine how long the SRP-specific antibodies remain detected in vaccinated birds and whether the antibodies are transferred to the progeny.

Several broiler breeder and layer flocks were vaccinated with *Salmonella* SRP vaccines as recommended. Blood samples were collected before and at multiple times after vaccination throughout the life of the flocks to monitor SRP antibodies over time. We also collected, incubated and hatched eggs from a breeder flock vaccinated with a newly licensed SE bacterial extract vaccine (Vaxxon® SRP® SE). The progeny chicks were raised in cages for 28 days and blood samples were collected weekly. All sera were assayed using the SRP ELISA to determine levels of SRP-specific antibodies.

These studies demonstrated that *Salmonella* SRP vaccines administered twice before lay were able to induce production of SRP-specific antibodies in vaccinated breeders and layers as detected by a proprietary *Salmonella* SRP ELISA. The SRP ELISA detected very low levels of SRP-specific antibodies prior to vaccination but showed a sharp increase in antibody levels post-vaccination. SRP antibodies were detected through 60 weeks of age in broiler breeders and egg layers. The SRP ELISA also demonstrated that the SRP-specific antibodies were transferred by the hens to their progeny which remained detectable till 14 days of age.

Key Words: siderophore receptor proteins, *Salmonella* Enteritidis, ELISA, SRP vaccines, SRP-specific antibodies

T159 Evaluation of administration route, volume, and formulation of an autogenous *Clostridium septicum* (CS) bacterin-toxoid on humoral response against CS α -toxin in commercial turkeys

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Clostridium septicum (CS) is the primary cause of Clostridial Dermatitis/Cellulitis in commercial turkey flocks. Due to the removal of antibiotic growth promoters and the consumer demand for no antibiotics ever (NAE) production, vaccination has been explored as an alternative solution to control Clostridial Dermatitis. Previously, we have shown that vaccination with a CS bacterin-toxoid combined with Seppic Montanide 71 R VG water-in-oil adjuvant (SM) increased humoral immune response to CS α -toxin, and reduced penicillin usage and CS-associated mortality in the field. Additionally, CS cellular growth phase and environmental fermentation conditions at time of formalin inactivation markedly influenced antigenicity and vaccine efficacy. The purpose of the present study was to evaluate the effect of vaccine administration route, volume, and formulation on antibody response to CS α -toxin. In the current study, CS bacterin-

toxoid produced under optimal fermentation conditions was combined with SM adjuvant at a ratio of either 50:50 CS/SM or 30:70 CS/SM. At 6 weeks-of-age, vaccine was administered subcutaneously (SQ) in the neck or intramuscularly in the caudal tail head (TH) at a 0.5mL or 1mL dose. Immunized treatment groups (n=20-21) included: 1) non-immunized control; 2) SQ neck, 0.5mL dose, 30:70 CS/SM; 3) SQ neck, 1mL dose, 30:70 CS/SM; 4) SQ neck, 0.5mL dose, 50:50 CS/SM; 5) SQ neck, 1mL dose, 50:50 CS/SM; 6) TH, 0.5mL dose, 30:70 CS/SM; 7) TH, 1mL dose, 30:70 CS/SM; 8) TH, 0.5mL dose, 50:50 CS/SM; and 9) TH, 1mL dose, 50:50 CS/SM. Blood samples were collected weekly from 6-12 week-of-age to measure serum antibody response to CS α -toxin. From 3-6 weeks post-immunization, groups 3, 5, 7, and 9 showed significantly ($P<0.05$) higher antibody levels, as measured by ELISA, compared to the non-immunized control. Groups 3 and 5 had the highest cumulative antibody response from 3-12 weeks post-immunization. From these results, the 30:70 CS/SM at 1mL SQ was the preferred combination. These findings suggest that formulation, dose, and route of administration are important factors to consider when implementing a vaccination program to combat Clostridial Dermatitis in commercial turkey flocks.

Key Words: dermatitis, cellulitis, turkeys, *Clostridium septicum*, vaccine

T160 The effects of possible alternative treatments for nematodes on egg production and quality Maria Tereza Bethonico Terra¹GS, Andrea Pietruska², Brett True¹, Kylee Hodgen³, Ruediger Hauck^{1,2} ¹Department of Poultry Science, Auburn University; ²Department of Pathobiology, Auburn University; ³Department of Animal Science, Auburn University

There has been an increase of market's demand for alternative production systems, which can increase challenges by endoparasites. Moreover, people are getting more concerned with the use of drugs in food animals, which urges the use of natural products. However, data on their efficiency as well as viability of their use is still limited. Our goal was to evaluate the effects of two potential alternative treatment for *Ascaridia galli* on egg production and quality.

Day-old layer-type birds were raised on the floor until 12 weeks of age when they were moved to cages. At 16 weeks, birds were divided into 3 treatments that were mixed in the feed: control, pumpkin seeds and artemisia extract. At 25 weeks, half of the birds were challenged with *A. galli*. Performance data was collected daily. As soon as birds reached 50% of production eggs were collected weekly for evaluation of quality.

The control group was the first to start lay eggs and maintained a slightly higher average of its weekly percentage of production until 23 weeks. However, differences were not statistically significant. After the challenge, all groups had lower egg production when compared to their respective non-challenged groups (differences were seen at different time-points). They were of 7.1%, 9.3% and 8.3%, for the control, artemisia and pumpkin groups, respectively. Treated groups had a significantly darker yolk color than challenged groups. Other egg quality analysis did not show significant differences. The average of Haugh units was 96.78% and, after the challenge, all groups were lower than the control non-challenge, but the differences were not enough to change the grade of that eggs that was always AA.

Challenged birds had lower production of eggs as well as egg yolks with lighter color. This suggests that the infection might interfere with birds' performance as well on its capability of absorption of pigments. Regarding feed treatments, no differences were noticed compared to the control group to this point. Continuation of data collection will offer more information about their efficiency against adult worms. Our findings suggest that the use of pumpkin seeds and artemisia can be administrated on layer hens without negative effects on egg production and quality.

Key Words: : Pumpkin seeds, Artemisia, Laying hens, Roundworms

T161 Cellular immune responses to avian *Clostridium perfringens* isolates Raveendra Kulkarni^{*}, Carissa Gaghan, Javid Mohammed *North Carolina State University*

Clostridium perfringens-induced necrotic enteritis (NE) is an economically important disease of broiler chickens. 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 and recent evidence suggests that there are unique NE-causing strains possessing virulence signature genes. Although NE pathogenesis is moderately well studied, avian cellular immune responses to *C. perfringens* and their secretory proteins is poorly understood. The present study used NE-producing virulent *C. perfringens* to evaluate their effects on primary chicken splenic and cecal tonsil mononuclear cells as well as macrophages (MQ-NCSU cells). The findings showed that stimulation of splenocytes and cecal tonsilloocytes with virulent *C. perfringens* bacteria or their secretory products resulted in a significantly increased frequency of macrophages coupled with an upregulated surface expression of MHC-II antigen-presenting molecule, as determined by flow cytometry. Additionally, the CD4⁺ and CD8⁺ T cell numbers as well as their MHC-II expression were also significantly elevated in *C. perfringens*-treated groups. Furthermore, MQ-NCSU macrophages stimulated with *C. perfringens* bacteria or their secretory proteins showed an augmented expression of IL-1 β , IL-6, IFN γ , CD80, CD40 and iNOS genes along with increased MHC-II surface expression and nitric oxide production. In summary, these results suggest that NE-causing *C. perfringens* isolates can induce robust T cell and macrophage responses characterized by their increased cellular frequencies and activation status.

Key Words: *Clostridium perfringens*, Necrotic enteritis, macrophages, cellular immune response, Chickens

T162 Evaluation of a nucleotide-rich yeast extract in a necrotic enteritis challenge model in broiler chickens Matthew Jones^{*1}, Charles Hofacre¹, Sergi Segarra² ¹Southern Poultry Research Group, ²Bioiberica SAU

Feed cost is the greatest expense to produce broilers so maintaining intestinal health is essential for the broiler industry. *Eimeria* species alone or with other intestinal insults degrade mucosal surfaces of the intestine and allow bacterial species, including *Clostridium perfringens* (CP), to proliferate. Dietary nucleotides modulate the immune response providing health benefits. This study evaluated the influence of a nucleotide-rich yeast extract (NU) product (Nucleoforce®, Bioiberica SAU, Spain) in a necrotic enteritis (NE) challenge when paired with a product containing an ionophore and a chemical anticoccidial (IC).

Five groups were represented by twelve replicates of 25 male broiler chicks: no-challenge, challenge control, IC, IC + NU at 500 ppm and IC + NU at 1000 ppm. All groups received coccidiosis vaccine at day of hatch. Challenged groups received CP on day 15, 16, and 17. On day 18, NE lesions were evaluated in 2 birds/pen and feces were collected for oocyst counts. Birds and feed were weighed on day 15, 35, and 42 to measure performance parameters. $P \leq 0.05$ was considered statistically significant.

All groups receiving IC had significantly lower oocysts/g of feces at 15 days. NE mortality was significantly higher in the challenged control compared to all other groups. NE lesion scores were lower in the unchallenged group compared to all challenged groups. Prior to the CP challenge, birds receiving NU had greater body weight gain and lower adjusted feed conversion ratio (aFCR) on day 15, compared to the IC group. After the challenge, the NU-supplemented groups maintained this significant advantage in aFCR at both 500 and 1000 ppm (1.47 in each), compared to the challenge control (1.58). The aFCR in the IC control was significantly lower than the challenge group and higher than the NU groups. The 0-42-day results were similar with lower aFCR in the NU groups compared to the IC group, and all of these challenged groups had lower aFCR compared to the challenged control.

Nucleoforce® supplementation may be useful in maintaining performance during periods of intestinal stress when used in combination with an ionophore program in broiler chickens.

Key Words: Eimeria, Clostridium Perfringens, Necrotic Enteritis, Nucleotide

T163 Duration of immunity of a recombinant HVT-IBD-ND vaccine against two different strains of velogenic NDV, and virulent and very virulent IBDV challenge Sing Rong, Kelly Turner-Alston, Candyce Pacione, Tura Bru, Lauren Taylor*, Rut Vila, John Dickson, Jennifer Embrey, Alicia Molas *Zoetis*

A recombinant HVT-IBD-ND vaccine was developed as a tri-valent vaccine for protection against infectious bursal disease (IBD), an acute and highly contagious viral infection of young chickens that causes immunosuppression and increased susceptibility to other infectious agents; Newcastle disease (ND), a highly contagious and fatal disease affecting all species of birds; and Marek's disease (MD), a common cause of condemnations and immune suppression in broilers and tumors in older birds. In one study with SPF leghorn chickens, recombinant HVT-IBD-ND vaccine was injected either in ovo at E18 or subcutaneously at hatch. On Day 63, birds from each treatment group were challenged with a velogenic NDV Texas GB. Protection of 97% (29/30) was observed for subcutaneously vaccinated treatment group, while 100% (28/28) protection was observed for the in ovo vaccinated treatment, with 0% (0/29) protection for control group. In a separate study with SPF leghorn chickens, recombinant HVT-IBD-ND vaccine was injected either in ovo at E18 or subcutaneously at hatch and challenged with a velogenic NDV Herts Weybridge 33/56 on Day 63. Protection of 100% (30/30) was observed for both treatment groups with subcutaneous or in ovo vaccination, with 0% (0/30) protection for control birds. In addition, a study was conducted to examine the duration of immunity by challenging the vaccinated birds with a virulent classic IBDV on day 63. One hundred percent (30/30) protection was observed for both treatment groups (subcutaneous or in ovo vaccination) of HVT-IBD-ND, with 7% (2/30) protection observed for control group. In a separate study, duration of immunity against challenge of a very virulent IBDV is being tested. The details of experimental design and study results will be presented.

Key Words: recombinant, HVT-IBD-ND, Newcastle disease, Marek's disease, infectious bursal disease

T164 Construction, stability and efficacy of a recombinant HVT-IBD-ND vaccine against IBDV, NDV and MDV challenges Sing Rong, Yugang Luo, Kelly Turner-Alston, Candyce Pacione*, Lauren Taylor, Tyler Brown, John Dickson, Jennifer Embrey *Zoetis*

Infectious bursal disease (IBD) is caused by the birnavirus IBDV. IBD is a worldwide problem, and all types of chickens in all regions are routinely vaccinated for the disease. Epitopes responsible for stimulating virus neutralizing antibody (VN) responses by the host are located on IBDV viral protein 2 (VP2). Newcastle disease virus (NDV) causes a highly contagious and fatal disease affecting all species of birds. NDV fusion protein (F) is one of the major viral glycoproteins present in the viral envelope and is the main immuno-protective NDV antigens. Marek's disease (MD) is a common cause of tumors, condemnations and immune suppression in chickens. The etiologic agent, serotype 1 Marek's disease virus (MDV), is a member of the family Herpesviridae. Herpesvirus of turkeys (HVT), is an avirulent turkey virus that is capable of replication in chickens. HVT has been demonstrated as a useful vector for delivering major avian antigens, as well as an effective vaccine for MDV. We constructed forty-three HVT-IBD-ND recombinants using various promoters, antigen sequences and poly A signals, and the target gene expression cassettes were inserted at various sites on the HVT genome. An HVT-IBD-ND recombinant vaccine was identified and selected for its excellent in vivo efficacy (100% protection, 30/30) at Day 28 in SPF (specific pathogen-free) birds against

a virulent classic IBDV challenge for both in ovo vaccinated and subcutaneously injected at hatch, with 0% (0/30) protection for control birds. In another study, challenged with a velogenic NDV, 95% (38/40) protection was observed for in ovo vaccinated birds, and 100% (40/40) protection was observed for birds vaccinated by subcutaneous injection, with 0% (0/40) protection for the control group. In addition, efficacy of this vaccine against a virulent MDV challenge was observed. Furthermore, recombinant vaccine stability was demonstrated utilizing PCR by in vitro passaging of a viral culture, as well as IFA and DNA sequencing. Recombinant vaccine stability was also demonstrated by PCR for viruses derived from in vivo passages.

Key Words: recombinant, HVT-IBD-ND, Marek's disease, Newcastle disease, Infectious Bursal disease

T165 Protection studies of recombinant HVT-IBD against early AL2 challenge in SPF broilers Kalen Cookson*, Manuel Da Costa, John Dickson, Jon Schaeffer *Zoetis*

AL2 is the most common IBD virus in U.S. broilers, accounting for as much as half of today's field isolates. Poulvac® Procerta™ HVT-IBD is a new single insert IBD recombinant vaccine—introduced in 2021. Previous studies in SPF leghorns reported at the 2020 AAAP showed this vaccine gave high levels of 14-day protection against classic IBDV (96-98%), vvIBDV (90-93%) and variant AL2 (78%). This paper will present two AL2 challenge studies conducted to measure early IBD protection in broilers. Study Design: Ross 708 broilers with no maternal antibodies to IBDV were given a full dose of Procerta HVT-IBD *in ovo* and raised in isolators (5 reps per treatment) until study termination. At 19 and 18 days, challenged birds received 3.5 and 3.0 EID50 by eye/nose drop (Studies 1 and 2, respectively). At 7 days post challenge, bursameter scores were recorded and bursa to body weight ratios (B:BW) were determined to calculate protection. Challenged birds with no bursal edema and a mean B:BW within 2 standard deviations of the non-challenged controls were considered protected. Results: Vaccinated birds had significantly bigger bursas based on mean bursameter scores (4.8-5.1) and B:BW (1.4-1.5) compared to the challenge controls (3.5-3.8 bursameter; 0.6 B:BW); and protection was 70% and 78%, respectively. Discussion: Conducting IBDV challenge studies in SPF broilers affords an excellent opportunity to measure early acquired protection from *in ovo* vaccination in broilers. In both studies, 95% of challenge controls had significant bursal atrophy, indicating full susceptibility and a solid challenge "take" at 18-19 days from 3.0-3.5 log10 of virus. There was no perceptible difference in challenge "take" and protection levels between the two studies. These results show that Poulvac® Procerta™ HVT-IBD can stimulate high levels of protection in broilers by 18 days of age—a time when maternal antibodies have significantly waned and field challenge can often get started.

Key Words: IBDV, Protection, Procerta, recombinant, AL2

T166 Comparison of Real-Time PCR and ELISA Surveillance for IBV in a Broiler Breeder Complex Brian Jordan*, Sunny Cheng, Eric Shepherd *The University of Georgia*

Infectious bronchitis virus (IBV) is an economically significant pathogen of commercial poultry. Clinical presentations of disease differ based on serotype of IBV, tissue tropism, type of bird (broiler, broiler breeder, or layer), and age of infection, and may range from inapparent to severe. A heavier focus is placed on IBV in broilers where infection can lead to airsacculitis and carcass condemnation at processing, which is a significant economic loss. Therefore, more diagnostic surveillance is performed on broilers to monitor IBV prevalence and infection. Conversely, the economic impact of IBV is often less severe in broiler breeders, causing drops in egg production and wrinkled eggs, and recovery is common. Because of these factors, routine IBV surveillance is not done to the same extent as in broilers. This lack of data creates a knowledge gap in understanding the efficiency of IBV vaccination in long-lived birds as well as the potential for

emergence of novel IBV serotypes. To begin assessing both parameters, we collected choanal cleft swabs for real-time PCR analysis and blood for ELISAs from multiple flocks of varying ages from a broiler-breeder complex in North Georgia. Flocks ranged in age from 6-60 weeks. Real-time PCR data of samples from flocks less than 11 weeks of age showed very little IBV present, indicating poor vaccination. Corresponding ELISA data shows very little IBV antibody, with the complex average falling well below the North Georgia average. After 13 weeks of age, real-time PCR data shows a heavier but more sporadic IBV infection, and ELISA titers confirm this with some farms being below average and some being well above. Additionally, several farms sampled were positive with the pan-

IBV real-time PCR assay but were not positive with any of the serotype specific assays, indicating a potential IBV variant present in these farms. Further sequencing and characterization of samples is ongoing. Interestingly, no clinical disease was noted in any of the farms sampled. Overall, analyzing real-time PCR data paired with ELISA titers complex wide provided a detailed picture of IBV vaccination and challenge. This data should be used to make improvements in vaccination protocols/practice and estimation of IBV challenge severity and timing.

Key Words: Infectious bronchitis virus, Real-time PCR, ELISA, Broiler breeder

Metabolism and Nutrition: General Nutrition

T169 Effects of DDGS inclusion in the feed on broiler performance, digestibility and gut health Gabriela Cardoso Dal Pont^{*1}, Cinthia Eyng², Cristiano Bortoluzzi³, Annah Lee¹, Yuhua Farnell¹, Michael Kogut⁴ ¹*Texas A&M*, ²*Western Parana State University*, ³*DSM Nutritional Products*, ⁴*USDA-ARS Southern Plains Agricultural Research Center*

Distillers dried grains with solubles (DDGS) contain a significant quantity of yeast biomass and insoluble fiber contents. Therefore, we designed an experiment to identify the effects of increased inclusion of DDGS in chicken feed on chicken performance, gut health, and digestibility. For this experiment, 144 male 1-day-old broilers were randomly distributed in 3 treatments with 8 repetitions of 6 chickens each. The treatments consisted of 0, 7, and 14% of DDGS inclusion in a based corn-soybean diet. The chickens were raised in metabolic pens from 1 to 28-d. The birds and feed were weighed at 7, 14, 21 and 28-d to calculate weight gain (WG), feed intake (FI) and feed conversion ratio (FCR). At 14 and 28-d, one bird per pen was euthanized and the intestine was collected for gut health evaluation throughout I See Inside (ISI) histologic methodology, and at 28-d ileal content was collected for digestibility calculation. Data normality was tested through the Shapiro Wilk test. The non-normal data were submitted to Kruskal Wallis test, while the normal data were submitted to ANOVA and compared using Tukey's tests, $P < 0.05$ was considered significant. No differences in the performance were observed at 7-d. Birds fed 7% DDGS presented greater WG than 14% DDGS at 14 and 21-d, similar to the control diet (0% DDGS). Also, 7% DDGS diet improved FI at 21-d, and FCR at 14 and 28-d compared to the 14% DDGS group. Feed with 14% of DDGS negatively affected FCR at 28-d compared to control. Birds fed 7% DDGS presented, at 14-d, decreased duodenal ISI score, indicating better health, but increased ileal ISI score compared to 14% DDGS group, indicating inferior ileal gut health. Inclusion of 14% DDGS improved gut status of duodenum of 28-d. Regarding digestibility, 7% DDGS group had a reduced dry matter and crude energy digestibility compared to control, but similar to 14% DDGS. Thus, the inclusion of 7% of DDGS might be beneficial to the broiler performance compared to standard corn-soy-fed birds. However, 7% of DDGS inclusion did not improve intestinal health

or ileal digestibility. Therefore, we hypothesize that 7% DDGS modulated intestinal microbiota increasing short chain fatty acids production which had a positive effect in the broiler's performance.

Key Words: gut health, ileal digestibility, broiler performance

Metabolism and Nutrition: Enzymes, Feed Additives

T170 Studies for risk assessment of recombinant live therapeutics Yiannis Kaznessis*, Kathryn Kruziki *General Probiotics Inc.*

We develop ProAspis100, a recombinant antimicrobial live therapeutic for prevention and control of necrotic enteritis associated with *Clostridia perfringens* in broiler chickens. ProAspis means defensive shield in Greek. The mode of action involves ProAspis100 being administered orally in chickens and reaching the upper GI tract (ileum and jejunum) of birds. ProAspis100 then expresses and secretes an antimicrobial peptide, which exhibits potent inhibition of *Clostridium perfringens* and is therefore expected to be responsible for the anti-*Clostridium* activity of ProAspis100. In 2020, we opened an Investigational New Animal Drug file with the Center for Veterinary Medicine at the FDA. CVM granted us waivers for sponsor fees, deeming our technology innovative. We tested ProAspis100 in an animal trial at an industry-recognized contract research organization and demonstrated the efficacy in a necrotic enteritis model. We also collected data related to the risk ProAspis100 poses to the environment and to human food safety. In terms of the environmental impact, we have found that ProAspis is a benign, non-pathogenic, non-virulent natural bacterial isolate, which is weakened by the deletion of a gene from its chromosome that is essential for growth. The antimicrobial activity is conferred by a plasmid that encodes for the expression and secretion of a small protein that is generally harmless to other organisms and degrades rapidly in any environment containing proteases. Based on the data, we concluded that the risk of accumulation of the organism or of the peptide in the environment is low. In terms of human food safety, we are focusing on 1) the safety of our antimicrobial new animal drug with regard to its microbiological effects on bacteria of human health concern (in accordance with GFI #152) and 2) the safety of residues of our drugs in human food (GFI #159). This study was conducted at Blue River Research with 112 floor pens and each with the dimension of 4' x 4'. This study employed the randomized block design. The study utilized 2576 commercial male broilers (Ross 708) from a nearby hatchery in NE floor pen challenge model. Each pen was an experimental unit. The study began on Day 0 (arrival) and ended on Day 42. There were eight treatment groups (one negative control without challenge, two negative controls with two different challenge levels, one positive control (BMD) with high level challenge, and two test article groups (GP1 and GP2) with two different challenge levels). Each treatment group used 14 pens, i.e. replicates. Each pen housed 23 birds on day 0. Treatment groups were represented as TG01, TG02, TG03, TG04, TG05, TG06, TG07 and TG08. There were fourteen blocks of eight pens and the treatment groups were randomly allocated to each of the eight pens within the block. A summary of data is as follows: • Lesion scores were significantly decreased in GP1 and GP2 treated groups. Furthermore, there was no statically significant differences in BMD vs GP-treated groups. • Mortality was significantly decreased in GP1 treated groups during Necrotic Enteritis phase. Similarly, there was no statically significant differences in BMD vs GP1-treated groups to reduce mortality. • ADG was increased in the GP-treated animals prior to challenge. • ADG was better in GP-treated than challenge during NE-phase of study (day 14-21). We compiled data from this study and communicated to FDA the following: -Modified live therapeutic do not survive well in the gut of chicken. -Only a small fraction is excreted unchanged in the environment. -Less than 1% of the organisms administered to chickens can be found overall in the gut contents of birds, on the surface of birds or in the floor litter. In this presentation, we will present the results of the animal study and detail the data that we have communicated to the FDA that support

our assessment that ProAspis100 poses a low risk to the environment and to human food safety.

Key Words: live therapeutics, necrotic enteritis, regulatory approval

T171 The effect of VANNIX™ C4 supplementation on turkey performance during a coccidial challenge Karen Vignale^{*1}, Greg Mathis², Brett Lumpkins³ ¹Kemin Animal Nutrition and Health, ²Southern Poultry Research, Inc, ³Southern Poultry Feed & Research, Inc

The objective of this study was to evaluate the effects of VANNIX™ C4 on turkey performance under a coccidiosis challenge for a 42-day (d) period. Performance and oocysts per gram of feces were used as key variables. Day old turkey poults were randomly assigned to 1 of 4 treatments (12 pens/treatment; 40 birds/pen): 1. Control, non-infected; 2. Infected control; 3. T2 + VANNIX™ C4, 0.75 lb./ton; and 4. T2 + zoalene, 125 ppm. On d14, all birds (except T1) received the coccidial inoculum diluted to a 1 mL volume. The inoculums were a mixture of field isolates of *Eimeria meleagridis* (50,000 oocysts/bird), *E. gallopavonis* (25,000 oocysts/bird) and *E. adenoides* (50,000 oocysts/bird). On d20 and d28, fresh fecal samples were collected from each pen to determine the degree of oocyst shedding/cycling. Oocysts per gram (OPG) were determined for each sample. Body weight gain (BWG), adjusted feed conversion ratio (FCR) and mortality were determined at d14, 20 and 42 of age. Statistical analyses were performed using JMP® (SAS Institute, Cary, NC) software and significance was determined at P≤0.05. No statistical differences were found at d14 and d20 on feed intake, FCR or BWG between the challenged groups (P>0.05). Adding VANNIX™ C4 significantly improved FCR at d42 when compared to the infected control (P<0.05; 1.66 vs. 1.70). Zoalene significantly improved FCR at d42 when compared to the infected control and to VANNIX™ C4, which was intermediate (P<0.05; 1.64, 1.70, and 1.66 respectively). Over the d0-42 period, BWG was significantly improved by VANNIX™ C4 when compared to the infected control (P<0.05; 2.47 kg vs. 2.40 kg). Zoalene had statistically similar BWG to VANNIX™ C4 (P>0.05; 2.5 kg vs 2.47 kg). Zoalene significantly decreased OPG at d20 when compared to the infected control (P<0.05); whereas, VANNIX™ C4 was intermediate. No differences on OPG at d28 were seen by any of the additives when compared to the infected control (P>0.05). VANNIX™ C4 significantly improved mortality at the d0-21 period and at the d0-42 period when compared to the infected control (P<0.05; 1.13% vs. 6.4% and 1.7 % vs. 4.4%, respectively). Overall, these results provide evidence that VANNIX™ C4 was able to improve performance of turkeys challenged with coccidia.

Key Words: coccidia, turkeys, performance, tannic acid extract, mortality

T172 Responses to high-fiber feed ingredients and dried fermentation product on growth performance, nutrient utilization, and gut microbiome of broiler chickens Chan Sol Park*, Tingting Wang, Carmen Wickware, Timothy Johnson, Olayiwola Adeola *Purdue University*

The aim of this study was to determine the influence of dietary prebiotic and high-fiber ingredients on growth performance, nutrient utilization, volatile fatty acid production, and gut microbiome of broiler chickens. On d 0 post hatching, 384 male broiler chickens [initial body weight (BW) = 45.7 ± 3.0 g] were assigned to 4 diets in a randomized complete block

design with BW as a blocking factor. Each experimental group consisted of 12 replicate cages with 8 birds per cage. Experimental diets were prepared as a 2×2 factorial arrangement with 2 diet types [i.e., low fiber (LF) and high fiber (HF)] and the addition of prebiotic in diets at 0 or 0.5 g/kg. Dried fermentation product (DFP) was used as a prebiotic. The LF diet was prepared based on corn and soybean meal, while the HF diet included wheat middlings, rice bran, and coconut meal. On d 0 to 7, broiler chickens fed HF diets had greater ($P = 0.008$) gain-to-feed ratio compared to those fed LF diets. On d 7 to 14, BW gain, feed intake, and gain-to-feed ratio of birds fed DFP were greater ($P < 0.05$) than those fed diets without DFP. The addition of DFP increased the apparent total tract metabolizability (ATTM) of dry matter and crude protein in LF diets, but not in HF diets (interaction $P < 0.05$). Birds fed LF diets had greater ($P < 0.001$) ATTM of gross energy and crude fat compared to those fed HF diets. The ATTM of gross energy in diets containing DFP was greater ($P = 0.004$) than in diets without DFP. The concentration of butyrate in cecal digesta of birds fed LF diets was greater ($P = 0.019$) than birds fed HF diets. The alpha diversity of bacterial community in both ileal and cecal digesta were not affected by either dietary fibrous ingredients or DFP addition. However, the beta diversity of bacterial community in cecal digesta was influenced by dietary DFP when estimated by unweighted UniFrac distances ($P = 0.037$). In conclusion, the addition of DFP in diets may increase the nutrient and energy utilization of diets, leading to an improved growth performance of broiler chickens. Dietary fiber contents influenced the utilization of nutrients and energy and production of butyrate in the ceca. Bacterial community in cecal digesta from broiler chickens was influenced by dietary DFP inclusion.

Key Words: broiler, dietary fiber, digestibility, microbiome, prebiotic

T173 Evaluation of a novel proprietary feed additive to strengthen or substitute avilamycin growth promoter in broiler standard program Angel-Rene Alfonso-Avila¹, Bertrand Medina², Ashley Wagner², Ivan Girard², Marie-Pierre Letourneau-Montminy³ ¹CRSAD (Centre de Recherche en Sciences Animale de Deschambault), ²Probiotech International Inc., ³Département des Sciences Animales, Université Laval

This study evaluated the capacities of Alterna®Poultry (AP), a blend of botanicals, organic acids and functional oils, as an alternative to antibiotic growth promoter or to strengthen avilamycin-based program. One-day old ROSS 308 chicks (n=2016) were randomly placed in 48 floor pens and assigned to 3 treatments: T1) Intepirity® @ 150 g/MT – 10% avilamycin during grower (d12 - 19) and finisher 1 (d20 - 29) phases, T2) AP @ 400 g/MT during starter (d1 - 11) and grower phases and 300 g/MT till the end and T3) a shuttle program equivalent to T1 plus AP @ 400 and 300 g/MT in starter and finisher 2 (d30-34) phases, respectively. At d4, a coccidia challenge with 15X Coccivac®-B52 (Merck) dose was applied on feed. Birds and feed were weighed at d11, 19, 29, and 33 to assess body weight (BW), daily growth (ADG), feed intake and feed efficiency (FCR). Litter moisture (LM) was measured at d11, 19 and 33. Foot pad dermatitis (FPD) lesions were scored at d21 and 32. Two birds per pen were euthanized at d32 to estimate apparent ileal digestibility of crude protein (AID CP) with insoluble ash as indigestible marker and to analyse plasma levels with VetScan® Avian/Reptilian Profile Plus. The data was analyzed using a mixed model with treatment as fixed effect. Comparisons of means were made with the Tukey test. Results showed that at d11, broiler BW fed with AP were greater (+2.6%; (T2, T3) vs T1, $P=0.02$). During grower, ADG was higher in T3 than T2 (+3.5%, $P=0.03$) while BW was higher for T3 birds (+3.5% vs T2, $P=0.001$). During the finisher phase 2, FCR was lower in T2 than T1 (-9%, $P=0.03$). Final BW from birds feed with AP was improved (+2.5%; (T2, T3) vs T1 $P=0.04$). At d32, plasma uric acid ($P=0.05$) and Na were lower ($P=0.03$) for T2 compared with T3, T2 albumin showed the lowest level ($P=0.03$), whereas T2 total protein tended to be lower compared with T3 ($P=0.07$). The highest AID CP was noticed for T2 (+5.5%, $P<0.001$). Both final LM ($P=0.03$) and FPD lesion score ($P<0.01$) were the lowest for T2. These results suggest that AP applied

alone or in shuttle program allows to achieve greater growth performances than avilamycin. Changes in plasma and AID CP also suggest a modulation of systemic acid-base balance and protein metabolism.

Key Words: Phytogenics, Broilers, Litter quality, Antibiotics growth promoters, Ileal digestibility

T174 Growth performance and cecal microbiome profile of broilers fed diets containing a macroalgae blend. Jason Sands^{*1}, Lauren Park², Douglas Currie², Augustine Owusu-Aseidu¹ ¹Ocean Harvest Technology Ltd., ²Roslin Nutrition Ltd.

Marine macroalgae contain relatively high levels of soluble polysaccharides and other bioactive compounds. The current study investigated the use of a marine macroalgae blend (MAB) comprised of green, brown and red species on growth performance and cecal microbiome profile in broiler chickens.

Ross 308 broilers (1120) were assigned to 2 dietary treatments, Control (C) or C with MAB at 5 kg/ MT of feed. Day-old chicks were randomly assigned to 32 cages, 35 birds per cage, providing 16 replicated cages per treatment. Chicks were allowed continuous access to pelleted, wheat and soybean meal- based diets that met or exceeded the requirements (NRC, 1994) for broiler chickens. Body weight, feed consumption and mortalities were recorded during the trial and body weight gain (BWG), and feed conversion ratio (FCR) calculated on completion of the trial. Ceca contents were collected by sacrificing 1 bird per cage at the end of the feeding trial. Ceca contents were used for DNA extraction, sequencing of the V3 - V4 variable regions of the 16S ribosomal RNA gene (Illumina, 2013 and 2014) and taxonomic profiling of the microbial community by BaseClear B.V. (<https://www.baseclear.com/>). Growth data were analysed using the ANOVA procedure for a completely randomised design with the Fit Model platform of Jump 14.2 (SAS Institute Inc, Cary, NC). Microbiome and growth data that did not follow a normal distribution, were analysed using the Wilcoxon non-parametric test. Tukey-Kramer test was used to compare means with significance declared at $P \leq 0.05$.

Added MAB significantly improved BWG over the 10-day starter period and tended to improve ($P=0.083$) overall. Adding MAB at 5 kg/ MT improved ($P=0.004$) FCR over the 42-day study period. Alpha diversity at family level was not different between groups. Based on relative abundance, at the highest taxonomic level, the cecal microbiome was comprised mostly of *fimicutes* (F), *Actinobacteria* and *Bacteroidetes* (B), with a F:B ratio of 17.7 and 22.6 for the C and MAB diets, respectively ($P>0.05$). The most abundant bacterial family was *Ruminococcaceae*, with a relative abundance of 10814 in the MAB group compared to 9391 in C ($P=0.14$). Improvements in BWG and FCR, suggests MAB may have prebiotic benefits in broiler chickens.

Key Words: Broiler chickens, Macroalgae, Growth performance, Cecal microbiome

T175 Dietary addition of a macroalgae blend improves growth performance and changes the gastrointestinal microbiota profile of commercial broiler chickens. Jason Sands^{*1}, Chongxiao Chen², Guanchen Liu², Augustine Owusu-Aseidu¹, Woo Kim² ¹Ocean Harvest Technology Ltd, ²Department of Poultry Science, University of Georgia

The effect of adding a Macroalgae Blend (MAB) to corn/soybean meal-based diets on growth performance and cecal microbiome profile was investigated in commercial broilers.

Day-old Cobb 500 chicks (864) were weighed and allocated to 36 floor pens, with 12 replicate pens of 24 birds. Birds were reared in an environmentally controlled room with 2:22 hour dark:light cycles and temperature maintained at breeder specifications. Nutrient adequate (NRC, 2004) mash diets, consisting of a Control (C) and C with MAB at 5 g/ kg or

10 g/ kg of feed, were fed in 3 phases, for 42 days. Diets were randomly allocated to pens and birds allowed free access to feed and water. Body weight and feed intake were recorded after each diet phase and mortalities daily. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated. Ceca samples were collected from 1 bird per pen for DNA sequencing of the V3 - V4 variable regions of the 16S ribosomal RNA gene (Illumina, 2013 and 2014) and taxonomic profiling of the microbial community (BaseClear B.V., <https://www.baseclear.com/>). Data were analyzed as a completely randomized design using the JMP 16.1 statistical program (SAS Inst., Inc., 2020-21), with pen as the experimental unit. Significant treatment effects were compared using contrast statements and Dunnett's test.

MAB linearly improved ($p=0.02$) body weight gain (BWG) and tended to improve FCR ($P=0.08$) in the starter phase (day 1-10). During the grower, finisher and overall periods, BWG, FCR and survival rates were not significantly different among groups ($p>0.10$). At the phyla level, the relative abundance of *Firmicutes* tended to increase ($p=0.06$) with added MAB. The *Firmicutes* to *Bacteroidetes* ratio, an indicator of a shift in microbiota profile, was 18.6 versus 23.2 in the control and MAB groups, respectively. At the family level, relative abundance of *Bacteroidaceae* tended to decrease ($p=0.08$) in birds fed diets with added MAB. The *Ruminococcaceae*, a key butyrate producing family in the *Firmicutes* phyla, accounted for over 37% and 41% of identified sequences ($p>0.10$) in the control and MAB groups, respectively. These results suggest adding MAB to broiler diets may improve performance by supporting the beneficial bacteria in the lower gastrointestinal tract.

Key Words: Broiler Chickens, Macroalgae, Growth performance, Cecal Microbiome

T177 Evaluation of the digestible calcium equivalency of a novel *Citrobacter braakii* phytase Carrie Walk¹, Pauline Jenn², Jingcheng Zhang³, Raffaella Aureli², Shikui Wang³, Anaëlle Tschambser², Zhenzhen Wang³, Qian Zhang³ ¹DSM Nutritional Products, ²DSM Nutritional Products, Research Center for Animal Nutrition and Health, ³DSM Nutritional Products, Animal Nutrition Research Center

Two experiments were conducted to estimate the digestible Ca (dgCa) equivalency of a novel *Citrobacter braakii* phytase when considering different levels of dietary phytate P, total Ca, and broiler age. In Exp. 1, Cobb 500 male broilers ($n = 768$) were housed in battery cages and fed experimental diets, containing 0.35% phytate P, from d10-12 or d22-24. The diets were arranged as a $2 \times 4 \times 2$ factorial, including low (0.60%) or high (1.0%) total Ca, 4 doses of phytase (0, 500, 1,000 or 2,000 FYT/kg) and two ages (d10-12 or d22-24). This resulted in 16 treatments with 6 pens/diet. In Exp. 2, Ross 308 male broilers ($n = 704$) were housed in battery cages and fed one of 8 treatments, containing 0.25% phytate P, from d8-10 or d21-24. Treatments included 4 concentrations of dgCa [0.22, 0.32, 0.42, or 0.52% (d8-10) or 0.16, 0.26, 0.36 or 0.46% (d21-24)] without phytase and 4 doses of phytase (350, 700, 1,400 or 2,800 FYT/kg) included in the lowest dgCa diet. This resulted in 16 treatments (8 diets \times 2 ages) with 8 pens/diet. In Exp. 1, Ca level \times phytase \times age influenced feed intake (FI, $P < 0.05$) and feed conversion ratio (FCR, $P < 0.05$); whereas body weight gain (BWG) was influenced by the main effect of age ($P < 0.05$) and linearly increased as phytase dose increased from 0 to 2,000 FYT/kg, but only in diets containing 1.0% total Ca (Ca \times phytase, $P < 0.05$). In Exp. 2, there was no effect of diet on FI, BWG or FCR. Apparent ileal digestibility of Ca was influenced by Ca \times phytase \times age ($P = 0.052$) in Exp. 1 or diet \times age ($P < 0.05$) in Exp. 2 and the dgCa equivalency for each phytase dose could be estimated using regression equations at each age. In Exp. 1 the estimated dgCa equivalency of 250 to 2,000 FYT/kg of phytase increased from 0.056 to 0.087% (d8-10) or 0.019 to 0.064% (d22-24), but only in birds fed 0.60% total Ca. There was no effect of phytase in birds fed 1.0% total Ca. In Exp. 2, the estimated dgCa equivalency of phytase was 0.083% at 250 FYT/kg and plateaued at 0.086% at 500 FYT/kg (d8-10); whereas, the estimated dgCa equivalency of 250 to 2,500 FYT/kg phytase increased from 0.013 to 0.041% from d21-24. The dgCa equivalency of phytase is between 0.013 to 0.087% and this can vary depending on the broiler age, dietary phytate or total Ca, and phytase dose.

Key Words: age, broiler, digestible calcium, phytase, phytate

Processing and Products

T179 Translocation and persistence of *Salmonella* in internal organs of broilers orally inoculated with varying doses of *Salmonella* cocktail Jinquan Wang*, Davis Fenster, Sasikala Vaddu, Sujitha Bhumanapalli, Thiago Belem, Anju Singh, Rami Dalloul, Jasmine Kataria, Gaganpreet Sidhu, Cortney Leone, Manpreet Singh, Harshavardhan Thippareddi *University of Georgia*

The transfer of *Salmonella* spp. to various internal organs of broilers orally inoculated with different doses of *Salmonella* cocktail over a 35d grow-

out period was evaluated. A total of 360 d-old chicks were placed to 18 pens of 3 groups (L, M and H) with 6 floor pens each. On d0, broilers were inoculated with a cocktail of *Salmonella* (equal population of marked serotypes; nalidixic acid resistant *S. Typhimurium*, rifampicin resistant *S. Infantis* and kanamycin resistant *S. Reading*) to create three groups: L: ~2 log CFU; M: ~5 log CFU; and H: ~8 log CFU. On d2, 7 and 35, 4 birds/pen were euthanized and ceca, liver, and spleen samples were collected aseptically. 4 gizzard samples/pen were collected on d35. All data were analyzed using SAS ANOVA with GLM procedure. A significance level was set at $p < 0.05$ and means were separated using Tukey's test. On d2, H group showed greater ($p < 0.05$) cecal colonization of all three serovars compared to L and M groups. However, M group showed greater ($p < 0.05$) colonization of all three serovars in liver and spleen compared to L group. The *Salmonella* colonization increased linearly in the ceca and quadratically in the liver and spleen with increasing of inoculation dose ($p < 0.05$). On d7, M group showed greater colonization of *S. Reading* in ceca compared to L and H group ($p < 0.05$). Similarly, M group had greater *S. Typhimurium* and *S. Reading* colonization in the liver compared to L group ($p < 0.05$). On d35, L group showed greater ($p < 0.05$) *S. Infantis* colonization in the ceca and liver compared to M and H group ($p < 0.05$). Moreover, within each group on d35, *S. Reading* counts were greater than those of *S. Typhimurium* and *S. Infantis* for all three doses for ceca and high dose for liver and gizzard ($p < 0.05$). *Salmonella* colonization diminished in ceca, liver, and spleen over time from d2 to 35 ($p < 0.05$). On d35, birds showed a similar total *Salmonella* spp. population in ceca (ca. 3.14 log CFU/g), liver (ca. 0.54 log CFU/g), spleen (ca. 0.31 log CFU/g), and gizzard (ca. 0.42 log CFU/g). Inoculation of *Salmonella* spp. at various doses linearly or quadratically increased the *Salmonella* colonization in organs at early age. The dose effect diminished with broiler age (D2 through d35).

Key Words: Broiler, *Salmonella*, movement, persistence, challenge

Metabolism and Nutrition: Enzymes, Feed Additives

T181 Effects of direct-fed microbial products on growth performance, intestinal morphology, ileal amino acid digestibility, litter moisture, and footpad dermatitis of male broilers. Rosana Hirai^{*1}, Austin Silva¹, Daniel De Leon¹, Garrett Powell¹, Macey Randig¹, Miloud Araba², Troy Lohrmann², Miguel Ruano², Audrey McElroy¹ ¹*Department of Poultry Science, Texas A&M University*, ²*Quality Technology International, Inc.*

The objective of the study was to evaluate the impact of feeding direct-fed microbial (DFM) products on growth performance, intestinal morphology (IM), ileal AA digestibility (IAAD), litter moisture (LM), and footpad dermatitis (FPD) of 42d old Ross 308 broilers. A total of 2,175 male chicks were obtained from a commercial hatchery and randomly allocated to 75 floor pens with used litter (0.085m²/bird). There were 5 treatments: Negative control (NC), no DFM; Bacitracin methylene disalicylate (BMD) at 50g/ton; Commercial single strain DFM product (C-DFM) at 500,000 cfu/g feed; a novel single strain Bacillus-based DFM product (Q-BioticTM 1DP) at 500,000 cfu/g feed; and a novel multi-strain Bacillus-based DFM product (Q-BioticTM 3DP) at 300,000/300,000/100,000 cfu/g feed. A randomized complete block design was used, and data were analyzed in SAS using the GLM procedure with significance at P<0.05. Fisher LSD's range test was used for mean separation. On d2, 14, 21, 28, 35, and 42, birds and feed were weighed to measure BW, BW gain (BWG), feed intake (FI), and FCR corrected for mortality. On d21 and 35, jejunum and ileum samples were collected for IM; and digesta samples were analyzed for IAAD. On d28 and 42, 5 samples of litter/pen were collected to measure LM. On d42, birds were evaluated for FPD lesions. For the cumulative trial period of d2 to 42, BMD and DFM diets improved FCR compared to the NC (P=0.0023). FI was highest for the BMD diet compared to diets with DFM inclusion (P=0.0045). There were no significant differences in final BW or cumulative BWG (d2-42). On d21, results demonstrated increases in villus height (VH; P=0.0008) and VH to crypt depth (CD) ratio (VH:CD) of jejunum (P=0.0180) when feeding BMD, C-DFM, or Q-BioticTM 3DP. There was a decrease in CD of ileum when feeding BMD or Q-BioticTM 3DP (P=0.0448). Feeding Q-BioticTM 1DP diets improved IAAD at d21 (P<0.0001). On d35, feeding Q-BioticTM 3DP diets improved CD (P=0.0194) and VH:CD (P=0.0305) of ileum as well as the IAAD (P<0.0001). No significant difference was observed for LM or FPD (P>0.05). The results suggest that these Q-BioticTM products could improve the growth performance, IM, and IAAD in broilers and may be an important factor of management programs for antibiotic-free poultry production.

Key Words: Q-BioticTM, Direct-fed microbial, Broiler performance, Intestinal morphology, Nutrient digestibility

T182 Phage Endolysins as Alternative Antibiotics to Control Clostridia in Poultry Michael Barnas^{*1}, Jennifer Timmons² ¹*AHPPharma, Inc.*, ²*University of Maryland Eastern Shore*

The endolysin PlyCP41 is known to exhibit lytic activity against *Clostridium perfringens* (CP) cells *in vitro*, and therefore could potentially be used *in vivo* to achieve similar results within the gastrointestinal tract. The objectives of this study were to confirm the lytic activity of PlyCP41 using a plate lysis assay and digesta collected from healthy broiler chickens at 28 days of age. Three different enzyme preparations of PlyCP41 were diluted to a starting concentration of 2 mg/ml then spot plated onto 11 ml of semisolid agar containing 1 ml of CP cells at 55 OD concentration. Each enzyme was serial-diluted then plated on a grid using 5 µl per spot in descending concentrations. The plates were incubated at 37°C for 2 h then observed for lytic activity. All three enzyme preparations lysed CP cells at 100 ng of enzyme per spot, and one purified protein preparation was effective at 10 ng per spot. Next, digesta was harvested and pooled by region (1. "Upper" containing crop, proventriculus, and gizzard; 2. The "small intestine" including the duodenum, jejunum, and ileum; and 3. Both cecal pouches) from three birds at 28 days of age. Contents were homogenized,

then three 1.0 ml aliquots from each area were assigned to two treatment groups, 1. A control group with lysis buffer only and no enzyme; and 2. Sonicated but unpurified PlyCP41 enzyme extract in lysis buffer. Each 1.0 ml aliquot of digesta was inoculated with 0.01 ml containing 6.74 log₁₀ CFU/ml CP and 0.05 ml of the control lysis buffer containing no enzyme or the lysis buffer containing PlyCP41 enzyme. Inoculated aliquots were incubated at 40°C for 1 hour. Samples were diluted and cultured on TSC agar for 48h at 37°C, and typical black colonies were counted to calculate the abundance of CP in each digesta sample. The control upper gut digesta contained 3.6 log₁₀ CFU/g CP, while the PlyCP41 samples contained undetectable levels of CP (<3 log₁₀ CFU/g). The small intestine contained 6.3 log₁₀ CFU/g in the control group and 4.9 log₁₀ CFU/g in the PlyCP41 group. Finally, the ceca contained 7.2 log₁₀ CFU/g in the control group and 7.1 log₁₀ CFU/g in the PlyCP41 group. Future studies will repeat this experiment using more replicates and concentrated/filtered PlyCP41 enzyme preparations.

Key Words: Necrotic enteritis, *Clostridium perfringens*, Bacteriophage, Enzyme, Endolysin

T183 Performance effects of either antibiotic growth promoters or functional oils in broilers challenged with coccidiosis Priscila de Oliveira Moraes¹, Paula da Silva Pires², Douglas Haese³, Carolina d'Avila Pozzatti³, Joan Torrent⁴ ¹*Dept. Zootecnia e Desenvolvimento Rural, UFSC*, ²*Dept. Zootecnia, UFRGS*, ³*Centro de Tecnologia Animal Ltda*, ⁴*Oligo Basics USA LLC*

A total of 2520 one-day-old male chicks (Cobb) were used in a completely randomized design with seven treatments, 12 pens/treatment, and 30 birds per pen to compare the effects of either different antibiotic growth promoters or a commercial mixture of functional oils (FO) on broilers challenged with coccidiosis. Treatments were: no additives (control), enramycin (8 ppm), virginiamycin (16.5 ppm), tylosin (55 ppm), and either 0.5, 0.75 or 1.00 kg/MT of FO (cashew nut shell liquid + castor oil; Essential, Oligo Basics, Cascavel, Paraná, Brazil). All diets were supplemented with 500 g/MT of Aviax Plus (3% semduramycin + 8% nicarbazin) from day 1 to 28 days of age and 500 g/MT of Coban 40 (sodium monensin 40%) from 29 to 42 days of age. At 14 days of age, 1 mL of a solution containing sporulated oocysts of *E. tenella* (10 × 10³), *E. acervulina* (200 × 10³), and *E. maxima* (80 × 10³) was inoculated by gavage to all birds. Performance parameters were evaluated at 21, 28, and 42 days of age. Statistical analyses were performed using an ANOVA, and a Tukey's test when necessary. The birds supplemented with tylosin were heavier (P < 0.05) and more feed efficient (P < 0.05) than the controls at 21 days, but no more differences were seen among the other treatments. At 28 d of age, only the tylosin supplemented birds were heavier (P < 0.05) than the other treatments. However, the feed efficiency was worse (P < 0.05) for the controls when compared to all the other treatments, and although the FO treatment at 0.5 kg/ton was better than the control, it was inferior to tylosin. Efficiency for the other treatments was intermediate. At 42 d, birds supplemented with tylosin were heavier and more efficient (P < 0.05) than all the other treatments. In conclusion, broilers challenged with coccidiosis did better when supplemented with tylosin than with any of the other treatments. Functional oils performed similarly to enramycin and virginiamycin when supplemented at 0.75 or 1.00 kg/MT.

Key Words: coccidiosis, functional oils, antibiotic growth promoters

T184 Evaluation of an encapsulated calcium butyrate on performance and necrotic enteritis mitigation in broiler chickens Jinquan Wang¹, Jundi Liu², Hanyi Shi¹, Amit Singh¹, Janghan Choi¹, Hanseo Ko¹, Luke Barnard², Brett Lumpkins³, Greg Mathis³, Woo Kim¹
¹University of Georgia, ²Eastman Chemical Company, ³Southern Poultry Feed & Research Inc

A 42-day trial was conducted to evaluate an encapsulated calcium butyrate (Ca-B) (GBM Performant by Eastman) on growth performance and necrotic enteritis mitigation in broiler chickens. A total of 2,000 one-day old Cobb-Cobb off-sex male broilers were placed on the floor pen with 50 birds per pen and 10 replicates per treatment. The study consisted of 4 treatments: non-challenged control, challenged control, challenged control + Ca-B low-dose (200/150/150 mg/kg for the starter/grower/finisher phase, respectively) and challenged control + Ca-B high-dose (350/250/250 mg/kg). All birds were sprayed with 1x commercial coccidia vaccine on day of hatch. Birds from the challenged treatments were inoculated in feed with ~5,000 oocysts of *Eimeria maxima* on d 14, followed by 10⁸ CFU/mL *Clostridium perfringens* on d 19, 20 and 21. Total pen and feed weights were assessed at the end of each phase on d 14, 28 and 42. On d 21 and 28, 3 birds per pen were randomly selected for intestinal lesion scoring. Results showed that birds from the challenged control had significantly higher cumulative FCR ($P < 0.05$) on both d 28 and 42 than the non-challenged control. On d 42, Ca-B treatments at both low-dose (FCR=1.664) and high-dose (FCR=1.647) significantly improved the cumulative FCR when compared to the challenged control (FCR=1.755). On d 21, both Ca-B supplemented treatments had significantly lower intestinal lesion scores compared to the challenged control ($P < 0.05$). However, no significant difference on lesion scores were observed 7 days post challenge (d 28) among all treatments. The claudin 1 gene expression in the jejunum on day 28 was significantly downregulated in the high-dose Ca-B treatment compared to the challenged control ($P < 0.05$). Adding Ca-B at both low-dose (200/150/150 mg/kg) and high-dose (350/250/250 mg/kg) was able to improve the cumulative FCR from d 1 to 42 and showed the beneficial effect on mitigating necrotic enteritis lesions in challenged broiler chickens.

Key Words: calcium butyrate, growth, coccidiosis, necrotic enteritis, broiler

T185 Effect of a proprietary plant extract-based product on weight gain, feed conversion, and intestinal lesions of Cobb 500 broilers during a mixed *Eimeria* challenge infection Cody Flores¹, Greg Mathis², Brett Lumpkins², Muhammed Shameer Abdul Rasheed¹, Kristy Dorton¹
¹Devenish Nutrition, LLC, ²Southern Poultry Feed and Research, Inc.

Two studies were conducted at Southern Poultry Feed and Research, Inc. (Athens, GA) to evaluate a proprietary plant extract-based product (EO; DeviSTAT Broiler NA, Devenish Nutrition, Fairmont, MN) on growth performance and intestinal lesions during an *Eimeria* challenge infection. One day old Cobb 500 broiler chicks (as hatched) were randomly allocated to battery cages with 6 replicates of 8 chicks per cage ($n = 144$ and 192 in study 1 and 2, respectively). In study 1, the 3 treatment groups were: uninfected-unsupplemented control (UUC), infected-unsupplemented control (IUC), and EO at 0.75 lb/ton. In study 2, the 4 treatment groups were: UUC, UIC, and EO at 0.5 or 1 lb/ton. At d 14, all birds except for UUC, were orally gavaged with 1 ml of a coccidia inoculum (100,000, 50,000, and 75,000 sporulated oocysts of *E. acervulina*, *E. maxima*, and *E. tenella*). Birds were fed a corn-soy based mash diet. Body weight and feed intake were measured on d 0, 14, and 20 to calculate weight gain and feed conversion ratio (FCR). On d 20, coccidiosis lesions were evaluated from 5 randomly selected birds per cage using Johnson and Reid (1970) method. Both studies involved a randomized complete block design with individual cage as the experimental unit. A one-way-ANOVA was used to evaluate the effects of treatments within *Eimeria*-infected groups. A separate contrast was used to compare UUC and IUC. Means were separated using Fisher's protected LSD and differences were considered significant

at $P \leq 0.05$. In both studies, growth performance was negatively ($P \leq 0.003$) affected and lesion scores were higher ($P \leq 0.003$) in IUC compared with UUC, which confirms a successful challenge. Broilers fed EO at either 0.75 (study 1) or 1 lb/ton (study 2) had better FCR ($P \leq 0.001$; study 1: 3.98 vs. 7.12; study 2: 1.89 vs. 2.25) from d 14 to 20 (challenge period) and minimized lesions ($P \leq 0.002$; study 1: 1.89 vs. 2.43; study 2: 1.46 vs 2.50) caused by *Eimeria* compared to IUC. In general, birds fed EO exhibited a dose-dependent improvement in FCR, where 0.5 lb/ton performed numerically better than IUC (2.07 vs 2.25), but 1 lb/ton provided the best FCR (1.89). Results showed that EO minimized the negative effects of an *Eimeria* infection on FCR and gut health, which could reduce economic losses.

Key Words: Broiler, Plant extract, *Eimeria*, Growth performance, Lesion score

T186 The effect of plant extracts, medium chain fatty acids, and their combination on broilers challenged with Necrotic enteritis. Brian Glover¹, Jose Charal¹, Milan Hruby¹, Michael Sims² ¹Archer Daniels Midland Animal Nutrition, ²Virginia Diversified Research Corp.

The current study evaluated the effect of two plant extracts (PE) products, medium chain fatty acids (MCFA), and the combination of these feed additives in diets fed to broilers challenged with *C. perfringens* (CP). The study consisted of a randomized complete block design with 2,520 Ross 708 broilers allotted into 7 dietary experimental treatments, each with 12 replicate pens of 30 birds/pen. Dietary treatments consisted of a control (Treatment 1; challenged broilers without feed additive), Treatment 2 (a combination of capsicum, carvacrol, and cinnamaldehyde, Xtract® 6930) at 0.1g/kg Treatment 3 (a combination of garlic tincture and eugenol, Xtract® Shield) at 0.1 g/kg, MCFA (Daafit® S) at 1 g/kg (treatment 4), Treatment 5 (half dose combination of Treatment 2 and 4), Treatment 6 (half dose combination of Treatments 3 and 4), and Treatment 7 (half dose combination of Treatments 2 and 3). Broilers were challenged on day 17, 18, and 19 with a broth consisting of CP added via water trough in each challenged pen. Additionally, at 4 days of age, used liter was added to each pen. No significant 42-day body weight or feed intake differences were observed among the treatments. Feed conversion ratio (FCR) was significantly ($P < 0.05$) improved in birds fed the combination of two PE products (Treatment 7), and numerically improved for combination treatments when compared to the challenged control treatment. Treatment 7 demonstrated a 7 point FCR benefit when compared to the infected control at 42 days of age ($P < 0.05$). A similar improvement was noted for mortality corrected FCR ($P < 0.05$) with broilers fed control diet demonstrating a 5 point increase to FCR when compared to broilers fed a diet containing a combination of PEs (Treatment 7). Lowest mortality (5.28%) was observed for broilers fed a diet containing a combination of PEs and MCFA (Treatment 6). The results of this study may demonstrate the opportunity for poultry producers to consider PEs and MCFAs alleviate performance losses associated with NE. Additionally, the results suggest that combining various feed additives may be more beneficial than relying on a single feed additive at lower doses.

Key Words: broilers, Necrotic enteritis, Plant extracts, MCFAs, challenge

T188 Natural prevention of coccidiosis and necrotic enteritis in experimentally infected broilers David Diez¹, Julia Pie¹, Anna Tesouro¹, Sudipto Halder², Amrita Dhara² ¹Biovet, S.A., ²AgriVet Consultancy P Ltd.

Coccidiosis and necrotic enteritis (NE) are two major diseases that affect poultry farming globally caused by *Eimeria* and *Clostridium perfringens* (CP), respectively. Pronutrients (PN) and Cimenol Ring (CR) are active molecules from plant extracts used to control these enteric infections, as well as to overcome the increased risk of drug resistance. PN are intended to prevent coccidiosis by reinforcing local gut immune system, while CR has a strong antimicrobial effect against CP.

A trial was conducted for 42 days to evaluate PN and CR as preventive tools for coccidiosis and NE, in which 640 one-day-old broilers distributed into 8 treatment groups and 8 replicate pens each. Treatments were an uninfected untreated control (NC) fed a diet without anticoccidials/antimicrobials; an untreated *Eimeria*-infected group (PC1); an untreated *Eimeria* and CP infected group (PC2). These groups were supplemented either with PN alone or with PN and CR together, which made a total of five supplemented groups: NC+PN, NC+PN+CR, PC1+PN, PC2+PN, and PC2+PN+CR. Differences with $P<0.05$ were set as statistically significant.

Body weight (BW) and feed conversion (FCR) were significantly worsened in PC1 and PC2 compared to NC. PN and CR supplementation improved BW and FCR in non-challenged groups compared to NC [uninfected] ($P<0.05$); and in challenged groups compared to PC1 and PC2 [infected] ($P<0.05$). PN alone significantly improved FCR compared to PC1; however, the improvement was not significant compared to PC2, suggesting that PN can reverse the effects of *Eimeria* challenge but the combination with CR is better when there is a greater enteric challenge. Litter moisture got increased in PC1 and PC2 compared to NC ($P<0.05$) due to the challenge. Challenged groups supplemented with PN and CR obtained similar ($P>0.05$) values than those of NC. Oocyst counts in PN and CR in the challenged groups were lower than PC1 and PC2 ($P>0.05$).

In summary, PN and CR are effective to prevent coccidiosis and NE. PN are highly effective against coccidiosis, though the combination of PN with CR is a better option when coccidiosis is complicated with NE. PN and CR are natural tools that do not leave residues, nor create resistances. Differences of gene expression were evaluated as well in the treatment groups.

Key Words: coccidiosis, necrotic enteritis, gut health, pronutrients, cimenol ring

T189 Effect of *Pichia guilliermondii* inactivated whole yeast cell product on performance parameters, gastrointestinal tract gross lesion scores, and gut permeability in broilers experimentally challenged with coccidiosis Mohamad Mortada*, Amanda Hesse, Annalise Anderson
ADM Animal Nutrition

A controlled challenge trial was conducted to evaluate the effect of *Pichia guilliermondii* inactivated whole yeast cell product (Citristim®, WYP) supplementation during coccidiosis. A total of 480 Ross 708 male broiler chicks were randomly assigned to five treatments. Treatments included an unchallenged control (negative control, NC), a challenged control (positive control, PC) and 3 challenged treatments supplemented with 0.25, 0.5 or 0.75 g/kg of WYP in corn-SBM basal diet. Treatments were replicated in 12 pens/treatment with 8 birds/pen in a completely randomized block design with two room blocks. On D14, NC birds received distilled water, while challenged birds were orally gavaged with 9×10^4 oocysts/bird of Coccivac B-52 vaccine containing live oocysts of *Eimeria acervulina*, *E. maxima*, *E. mivati*, and *E. tenella*. On D21, 1 bird/pen was gavaged with 2.2 mg/ml/bird of fluorescein isothiocyanate-dextran (FITC-D). After 2 h, birds were euthanized, and blood was collected for measuring FITC-D levels in serum. On D22, a total of 12 birds/treatment were euthanized for gross lesion scores in the upper and middle GIT, and ceca. Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were analyzed for the starter (D0-14), grower (D15-28) and finisher (D29-42) phases and overall (D0-42). There was no treatment effect on BWG, FI or FCR during the starter phase (pre-challenge). Coccidiosis challenge significantly induced a 214 g reduction in BWG and a 240 g reduction in feed intake during the grower phase ($P<0.01$). In addition, coccidiosis challenge increased D22 lesion score severity in the middle GIT and ceca and D21 gut permeability when comparing the PC to the NC ($P<0.05$). On D21, supplementation of 0.25 g/kg WYP had lower serum FITC-D levels compared to the PC ($P<0.05$) indicating a reduction in gut permeability. On D22, 0.75 g/kg WYP treatment had less severe lesion scores in the

middle GIT and ceca compared to the PC ($P<0.05$). Overall, there were no treatment effects on overall BWG or FCR. However, there was a tendency in overall FI ($P=0.09$) with 0.25 g/kg WYP reducing feed intake by 126 g compared to the PC (4,630g Vs. 4,756g, $P=0.19$). In conclusion, WYP may assist in alleviating coccidiosis in poultry.

Key Words: Coccidiosis, *Pichia* yeast, gut permeability, lesion scores, performance parameters

T190 Effect of dietary supplementation of essential oil as an alternative to the use of antibiotic growth promoters in laying hens Swanny Ramirez^{1,2}, Maria Ospina¹, Edgar Santos³, Paola Amaya-Hernandez¹, Julieth Ducuara-Suarez¹, Lina Peñuela-Sierra^{1,2} ¹Universidad del Tolima, ²Grupo de Investigación en Sistemas Agroforestales Pecuarios, ³NutrianSA

Transition to antibiotic-free broiler production has driven the need for non-antibiotic solutions for disease challenges that affect animal performance for that has been considered is the implementation of Essential Oils due to their antimicrobial properties. The objective was to evaluate the effect of the oregano essential oil (OEO), on performance and egg quality parameters as an alternative to the use of antibiotic growth promoters (AGP). A total of 96 laying hens (70 weeks old) were randomly distributed in 4 treatments, with 4 replicates and 6 birds per replicate. The four treatments were: NC: negative control without OEO and AGP, AGP: diet with 50 ppm Zinc Bacitracin, 80OEO: diet with OEO 80 ppm, and 150OEO: diet with OEO 150 ppm. The hens were supplied with the diets for a total of 10 weeks, 2 as an adaptation and 8 for data collection. Data were analyzed with ANOVA and differences considered significant at $P<0.05$. The performance were recorded daily. Measurements of the internal and external egg quality were made using three eggs per replicate. The 150OEO treatment group had a higher feed intake than all other treatment groups ($P<0.05$) and a higher percentage egg production than the NC group ($P<0.05$). However, there were no differences in the weight gain of the birds or the average weight of the eggs among the treatments. The OEO and GPA treatment groups had better feed conversion ratios than the NC group ($P<0.05$). No differences were found in the % of yolk, albumin, or shell among the treatments ($P>0.05$). However, the 150OEO treatment had a greater albumin height and haugh units value than the NC and GPA groups ($P<0.05$), and both OEO treatment groups had higher yolk color scores than the NC and GPA groups ($P<0.05$). In terms of the external egg quality, the shell thickness was greater for the 150OEO and 80OEO treatment groups than for the NC and GPA groups ($P<0.05$), but the breaking force did not show differences among the treatments ($P>0.05$). The OEO treatment groups also had lower values of L and higher values of a* and b* for the color of the shell than the GPA and NC groups ($P<0.05$). The findings of this study indicate that 150 ppm OEO can be used as a substitute for AGP to improve the performance and egg quality parameters in laying hens.

Key Words: Essential oil, Oregano, laying hens, egg production

T191 Effect of a precision glycan in broiler chickens under a necrotic enteritis challenge Cristiano Bortoluzzi*, Joshua Claypool, April Levy, Shelby Corray, Jose Sorbara, Ghislain Schyns DSM Nutritional Products

The objective of the present study was to evaluate the effect of a novel precision glycan microbiome metabolic modulator (MMM) on the growth performance and the metagenome of the cecal microbiome of broiler chickens undergoing a mild necrotic enteritis (NE) challenge. Day-old chicks were placed on a completely randomized block design with 3 treatments, 10 replicates, and 25 birds/replicate. The treatments consisted of a non-supplemented and non-challenged control (CON), a challenged control (ChaCON), and a challenged group supplemented with 400 ppm of MMM precision glycan. The challenge consisted of a coccidia vaccine (Coccivac B52) at d 0 applied to all treatments, and *Clostridium perfringens* strain #6 was given via drinking water to the challenged groups on

d 15, 21, and 28. Growth performance parameters was evaluated on d 14, 28, and 45. NE lesions were evaluated on d 16. On d 22 and 46, 1 bird/pen was randomly selected, and the cecal content was collected for microbiome analysis. Regarding the growth performance of the animals from d 0 to 45, the challenged significantly reduced the body weight gain ($P = 0.02$) and worsened the feed conversion ratio (FCR; $P = 0.001$). The supplementation of MMM precision glycan totally recover the FCR of the birds, similar to the CON birds, and reduced the NE associated mortality ($P = 0.0002$). Changes in the microbiota at d 22 showed a decrease in *Butyrivibrio pullicaecorum* in ChaCON ($P = 0.06$) and MMM supplemented birds ($P = 0.10$). Within the ChaCON group, a minor increase in *Escherichia coli* at d 22 was also observed but the increase was not present in the MMM supplemented birds. At d 46, a decrease of *Barnesiella intestinihominis* was noted in the ChaCON group relative to both the MMM ($p = 0.03$) and the CON, while the challenge increased ($p = 0.10$) *Parabacteroides johnsonii*. Using Songbird, a tool to understand compositional data, the MMM supplementation appeared to restore several microbial pathways affected by *C. perfringens* including nitrogen, propionate, butyrate, fatty acid biosynthesis, and biosynthesis of cofactors. Some pathways suggest a link towards modulation of the immune response. Further investigation is needed to understand how the MMM impacts the host.

Key Words: broiler, microbiome metabolic modulator, necrotic enteritis, nitrogen metabolism

T192 M52, a novel natural feed additive, preserved mucosal immune and intestinal microbial homeostasis of broilers challenged with *Eimeria* spp. Hongyu Xue¹, Fernanda Rigo², Breno Beirão², Celso Fávaro², Max Ingberman² ¹Amlan International, ²Imunova Análises Biológicas

M52 is a natural anticoccidial feed additive featuring a select blend of bioactive phytochemicals. Recent research suggests the formulation has potential as an alternative to ionophores and chemical coccidiostats to help prevent coccidiosis while improving gut health and maintaining bird performance. This study further explored the potential effects of the product on host coccidial immunity, the composition and structure of the gut microbiome, and intestinal integrity of broilers challenged with experimental coccidiosis.

In this 28-day study, day-old Cobb broiler chicks were randomly assigned to 1 of 3 groups: 1) unchallenged control; 2) *Eimeria*-infected control; and 3) *Eimeria*-infected + M52 (70g/MT feed). On d14 chickens from Treatment 2 & 3 were challenged with 100X Bio-Coccivet R Vaccine consisting of sporulated oocysts of seven *Eimeria* spp. Peripheral blood mononuclear cell phenotype, ceca-cecal tonsil cytokine mRNA expression, gut microbiome of cecal content and duodenal/jejunum histopathology were examined.

M52 markedly alleviated *Eimeria*-induced histopathological changes, i.e., prominent villus hyperplasia, heterophil mucosal infiltration, and hemorrhagic or necrotic foci on d19. On d28, M52 treatment better preserved the competence of protective mucosal immunity by significantly increasing the abundance of mucosal cytotoxic (CD4-TCRVβ1+) and helper (CD4+TCRVβ1+) T cell and immune-responsive CD8-CD28+ helper T cell subsets (vs. *Eimeria*-infected control, $P < 0.05$). Further, on d19, M52 dampened *Eimeria* challenge-associated upregulation of cecal IL-10 (vs. *Eimeria*-infected control, $P < 0.01$), which may help overcome parasite immune evasion. *Eimeria* challenge significantly decreased the α -diversity of the cecal microbiome (vs. unchallenged control, $P < 0.05$), which was completely normalized with M52 treatment (vs. *Eimeria*-infected control, $P < 0.05$). Furthermore, M52 conferred challenge-independent protection to the intestinal homeostasis via promoting the relative abundance of *Blautia* and *L-Ruminococcus* genera, two short-chain fatty acid producers.

Collectively, M52 treatment promoted a well-balanced immune homeostasis, dampened intestinal damage, and preserved the microbiota diversity, which all contribute to an enhanced resilience to *Eimeria* spp. challenge.

Key Words: coccidiosis, anti-*Eimeria* immunity, IL-10, microbiome, intestinal integrity

T193 Efficacy and phosphorus equivalency values are greater for a novel bacterial phytase compared with a commercial bacterial phytase in broilers from 8 to 21 days of age Qian Zhang¹, Jose Otavio Sorbara², Carrie Walk², Kostas Stamatopoulos² ¹DSM China Animal Nutrition Research Center Co., Ltd, DSM Nutritional Products China, ²DSM Nutritional Products, Animal Nutrition and Health

The objective of this study was to determine the P equivalency of two bacterial phytases derived from *Citrobacter braakii* in the diets of broiler chickens from d8 to 21 post-hatch. Ross 308 male broilers were obtained at hatch and fed a common corn and soybean meal diet, deficient in total Ca (0.67%) and non-phytate P (nPP, 0.20%) until d7 of age. On d8, 576 broilers were weighed and allocated to one of 12 treatments until d21. There were 8 birds/cage and 6 pens/diet. The 12 diets consisted of 4 levels of nPP and total Ca (0.20 and 0.67, 0.28 and 0.79, 0.36 and 0.91, or 0.45 and 1.03%, respectively) and 4 doses (250, 500, 1,000 or 2,000 U/kg) of a novel bacterial phytase (HiPhorius) or a commercially available bacterial phytase (HiPhos). The phytases were supplemented into the diet containing 0.20% nPP and 0.67% total Ca. The diets were corn and soybean meal, pelleted, and provided ad libitum. Phytase recoveries in the diets were between 88 to 146% of the expected values. Decreasing the dietary concentration of nPP and Ca decreased feed intake (linear $P < 0.05$), weight gain (linear, $P < 0.05$), tibia ash weight (linear, $P < 0.05$) and tibia ash percent (linear, $P < 0.05$) and increased feed conversion ratio (linear, $P < 0.05$). Supplementation of the low nPP and Ca diet with increasing concentrations of HiPhorius or HiPhos increased feed intake (quadratic, $P < 0.05$), weight gain (quadratic, $P < 0.05$), tibia ash weight (linear, $P < 0.05$), and tibia ash percent (linear, $P < 0.05$) and improved feed conversion ratio (quadratic, $P < 0.05$). HiPhorius supplementation at 2,000 U/kg improved tibia ash weight and percent above that of the highest nPP and total Ca diet. Therefore, the P equivalency values of the two phytases were estimated using regression equations and the lower 95% confidence intervals for all parameters. The mean P equivalency values for 250, 500, 1000 or 2000 U/kg of HiPhorius were estimated at 0.116, 0.170, 0.211 or 0.235%, respectively. The mean P equivalency values for 250, 500, 1000, or 2000 U/kg of HiPhos were estimated at 0.097, 0.147, 0.188 or 0.213%, respectively. The results of this study showed the novel phytase (HiPhorius) was more efficacious compared with HiPhos in hydrolyzing phytate for growth and bone mineralization of broilers from d8 to 21 post-hatch.

Key Words: Phytate, Performance, Phytic Acid

T194 Supplementation of a novel consensus bacterial 6-phytase variant in diets reduced in nutrients and energy and with no added inorganic phosphorus maintained egg productivity and egg quality A. Bello¹, E. White¹, Y. Dersjant-Li¹, D. C. Z. Donato², T. F. Moura³, M. Reis³, N. K. Sakomura³ ¹Danisco Animal University, IFF, Netherlands, ²Danisco Animal University, IFF, Brazil, ³School of Agricultural and Veterinarian Sciences, São Paulo State University (UNESP) Jaboticabal

The efficacy of a novel consensus bacterial 6-phytase variant (PhyG) to alleviate the adverse effects of dietary nutrient and energy reduction and with no added inorganic P (IP) or bone meal was evaluated based on egg productivity, shell quality, and BW of laying hens from 23 to 46 weeks of age (woa). A total of 336 Hy-Line W80 (4 hens/cage \times 28 cage replicates per treatment) were randomly assigned to three dietary treatments. The diets were 1) a nutrient and energy adequate positive control diet (PC), 2) a nutrient and energy reduced negative control diet with reduction of 0.20% Ca, 0.02% Na, up to 0.04%-unit dig. AA and 75 kcal/kg ME vs PC and without supplementation IP or bone meal ingredients (contained 0.12% of

available P: NC), and 3) NC diet supplemented with 600 FTU/kg PhyG (NC+600G). The diets were based on corn-SBM with wheat bran and rice bran and were fed *ad-lib* through 16 hours of daily light. Data were analyzed using JMP 15.1 and treatment means separation by Tukey test ($P < 0.05$ as significant). Marketable egg production (23 to 46 woa) was improved ($P < 0.05$) for NC+600G (95.7%) vs NC (93.5%), while PC showed intermediate response (94.5%) and diet effects on average daily feed intake per hen, egg mass, and eggshell thickness followed the same pattern ($P < 0.05$). Eggshell breaking strength across the 23 to 46 woa was decreased ($P < 0.05$) by the NC (4.65 kgF) vs PC (5.07 kgF), however fully alleviated by NC+600G (5.10 kgF). Similarly, average BW (measured on eight weeks basis) was decreased ($P < 0.05$) by the NC (1,523 g) vs PC (1,576 g), however fully alleviated by NC+600G (1,567 g). Relative to

the PC, the NC+600G lowered feed cost / dozen eggs produced (0.538 vs 0.514 USD). While the NC hens were resilient in maintaining egg production (vs the PC hens) using their body reserve, the supplementation of PhyG to the NC cost-effectively enabled the hens on the nutrient reduced and IP/bone meal-free diet to better utilize dietary nutrients for egg productivity and maintaining high eggshell quality. In conclusion, in peak lay period, PhyG was able to totally replace inorganic P, and compensate for reduction of Ca, Na, dig AA and energy, maintaining egg production and quality.

Key Words: egg production, inorganic phosphorus-free, laying hens, phytase, shell quality

Metabolism and Nutrition: Enzymes, Feed Additives

T195 Supplementation of a novel consensus bacterial 6-phytase variant at 750 and 1,500 FTU/kg cost-effectively maintained growth performance of broilers fed corn-SBM diets from 1-42 days of age Katie Hilton¹, Abiodun Bello², Yueming Dersjant-Li², Eric Sobotik³, Gregory Archer³, Franco Mussini², Janet Remus² ¹Danisco Animal Nutrition, IFF, ²Danisco Animal Nutrition, IFF, ³Texas A&M University

This study evaluated the efficacy of a novel consensus bacterial-6 phytase variant (PhyG) supplementation at 750 and 1,500 FTU/kg on growth performance of broilers fed typical corn-SBM diets with respective nutrient and ME matrices application. A total of 1,080 male broilers (27 birds x 10 floor pens a treatment) were randomly allocated to each of four dietary treatments in a randomized complete block design. The four diets tested in this study were 1) a nutrient adequate diet (PC); 2) a diet reduced by 0.164% in dig P, 0.201% in Ca, 0.037% in Na, up to 0.035% unit in dig AA and 29 kcal/kg in ME vs PC and supplemented with 750 FTU/kg PhyG; 3) a diet reduced by 0.19% in dig P, 0.23% in Ca, 0.05% in Na, up to 0.045% unit in dig AA and 37 kcal/kg in ME vs PC (NC); 4) diet 3 supplemented with 1,500 FTU/kg PhyG. In addition, all diets had 2,000 XU xylanase supplementation with a 25 kcal/kg ME matrix application. Pelleted diets were based on corn/SBM with <2% DDGs and were fed *ad libitum* over four phases; starter (d1-10), grower (d11-21), finisher 1 (d22-35), and finisher 2 (d36-42). Feed intake and bodyweight were measured at d1, 10, 21, 35, and 42 to calculate body weight gain (BWG), average daily feed consumption (ADFC), and mortality corrected feed conversion ratio (FCRc). All data was analyzed by One-way ANOVA using JMP Pro 15.1 (SAS Institute, 2019). Treatment means were further separated using Tukey's HSD test. PhyG treatment at each dose-level produced growth performance measures that were by phase and cumulatively equivalent to PC. No differences in ADFC was seen however day 0-42 BW and BWG was 200g more ($P < 0.05$) when compared to NC. Furthermore, d0-21, d0-35 and d0-42 showed NC2 had the lowest ($P < 0.05$) FCRc compared to NC by 7, 7, and 8 points, respectively. This study indicates utilizing the matrix for 1500FTUs was most efficient for BW, BWG, and FCR.

Key Words: Broiler, phytase, corn-SBM

T196 A novel consensus bacterial 6-phytase variant maintained performance in broilers fed corn-SBM diet without added inorganic P and deficient in nutrients and energy Leon Marchal^{1,2}, Abiodun Bello¹, Yueming Dersjant-Li¹, Janet Remus³, Eric Sobotik⁴, Gregory Archer⁴ ¹Danisco Animal Nutrition (IFF), ²Wageningen University & Research, ³Danisco Animal Nutrition, IFF, ⁴Texas A&M University

Recent studies reported that a novel consensus bacterial 6-phytase variant (PhyG) supplemented to all plants based complex diets containing corn, wheat, soybean meal, rapeseed meal and rice bran with high phytate P content (>0.33% in starter), was able to totally replace inorganic phosphate (P) in all feeding phases in broilers. Current study tested this

approach in a simple diet containing corn and soybean meal (with corn DDGs at 1.5 to 2.2%) and with application of nutrients and energy down spec. A nutrient adequate, but not over-specified, 4 phased (0-10, 10-21, 21-35, 35-42 days) positive control (PC) diets were formulated with dietary phytate P level (based on the phytate P analysis in the ingredients) gradually reduced from 0.28% in starter phase to 0.25% in finisher 2 phase. The negative control (NC) was formulated with reduction of dig P (-0.19%), Ca (-0.23%), Na (-0.045%), dig AA (up to 0.05% unit for most limiting AA) and ME based on the expected contribution of PhyG at 1500 FTU/kg. The NC was further formulated as inorganic P free and supplemented with PhyG at 3000, 2000 and 1500 FTU/kg in starter, grower and finisher 1&2 phase respectively (IPF+PhyG). All diets contained xylanase at 2000 U/kg (with ME reduction considering xylanase or the combination of phytase and xylanase). Each treatment diet was pelleted and fed *ad lib* to 10 replicated pens (Ross 308 male broilers; 27 birds/pen). Data were analyzed by one-way ANOVA using JMP 14, treatment means were separated by Tukey test ($P < 0.05$ as significant). In each and overall feeding phases, the NC treatment decreased performance ($P < 0.05$) compared to PC. The IPF+PhyG maintained performance comparable to PC in all feeding phases. The 42d BW was 2888, 2684 and 2887g and overall mortality corrected FCR was 1.62, 1.72 and 1.62 respectively in PC, NC and IPF+PhyG groups. This indicates that even in a vegetable corn soy diet the without added inorganic P or meat and bone meal the phytase was able to compensate the nutrients /energy reduction and released sufficient P to meet the requirement. Feed cost /kg BWG was reduced in IPF treatments vs PC. This study showed that the new phytase has the potential to replace inorganic P in all feeding phases in broilers fed a corn and soybean meal-based vegetable diet.

Key Words: broilers, corn SBM-diet, phytase, inorganic phosphate free, performance

T197 Accumulation of fumonisins in broiler tissues with or without feeding algae-clay based technology Julia Laurain¹, Didier Tardieu², Maria Matard-Mann¹, Maria Angeles Rodriguez¹, Philippe Guerre² ¹Olmix Group, ²National Veterinary School of Toulouse, ENVT, University of Toulouse

It was long considered that fumonisins (FB) don't accumulate in animals as they have a low oral absorption and a rapid plasma elimination. Nevertheless, recent studies demonstrated that the hepatic half-elimination of fumonisin B1 (FB1) was up to several days. Based on these findings, the aim of this study was to evaluate fumonisin deposit in broiler tissues. Twenty-one-day old broilers were slaughtered after being fed a diet with 20 mg FB/kg for 4 or 9 days prior to slaughtering at 21 days, with or without an algae-clay based technology (Olmix, France). The exposure to fumonisins alone showed no sign of toxicity on broilers performance at this level of FB in feed, but increased concentrations of sphinganine (Sa) and sphingosine (So) over time were measured in the liver. An accumula-

tion of FB1 was measured in the liver, with concentrations of 20.3 and 32.1 ng FB1/g observed respectively after 4 and 9 days of exposure. The same pattern was observed with fumonisin B2 (FB2), with hepatic levels of 0.79 ng/g after 4 days and 1.38 ng/g after 9 days of exposure. Even if FB1 levels were very low in breast muscles, an accumulation could also be observed after 4 and 9 days of exposure with concentrations of respectively 0.036 and 0.072 ng FB1/g. When feeding the algae-clay based technology to the broilers, a reduction of FB1 deposit in the liver and muscles was measured. After 9 days of exposure, a significant reduction of around 40 and 50% was observed, whereas a non-significant effect was observed after 4 days exposure. Broilers fed the algae-clay based technology also showed a decrease in Sa and So levels in the liver compared to the non-supplemented broilers. Further studies are needed to confirm this fumonisin accumulation over longer time of exposure and different animal age. Meanwhile, the algae-clay technology shows promising results to prevent such deposit.

Key Words: fumonisin, broiler, liver, muscle, feed

T198 Effect of supplementation of a mycotoxin mitigation product on performance and aflatoxin metabolism in broilers fed mycotoxin contaminated diets Sangita Jalukar¹, Yong Fang², Feifei Han², Yutong Fu², Jinglin Ma², Lihong Zhao² ¹*Arm and Hammer Animal and Food Production*, ²*State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University*

A study was conducted to test the effects of supplementation of a mycotoxin mitigation product on performance and aflatoxin metabolism in broilers fed diets contaminated with mycotoxins. One-day-old Arbor Acre male broilers received one of six different treatments made up of two diets and three inclusion rates of test product arranged in a 2 x 3 factorial design. The trial was conducted in eight replicate cages per treatment with 10 birds per cage for 42 days. The mycotoxin mitigation product (BG-MAXTM, Arm and HammerTM) was fed at 0 kg/MT, 1 kg/MT and 2 kg/MT. The control diet was formulated with clean corn and the mycotoxin diet was formulated with moldy corn (Aflatoxin B1 (AFB1) 130 ppb, Deoxynivalenol (DON) 1.5ppm, Zearalenone (ZEN) 496ppb). Performance of the broilers was measured at the end of the trial. Liver samples were collected on d42 to measure AFB1 metabolism and deposition of AFB1. The data were analyzed statistically with significance noted at $P < 0.05$. Body weight (BW) was reduced, and feed conversion ratio (FCR) was increased in broilers fed mycotoxin contaminated diets compared to those fed the control diet or mycotoxin contaminated diet but supplemented with BG-MAX. Expression of three of the four key enzymes of the cytochrome P450 enzyme system in the liver were upregulated when broilers were fed mycotoxin contaminated diets without BG-MAX. Supplementation with BG-MAX showed reduction in expression of cytochrome P450 enzyme P1A2 compared to broilers fed mycotoxin contaminated diets without BG-MAX. Expression of Glutathione transferase (GST) enzyme was reduced and that of DNA repair enzyme p53 was increased in broilers fed mycotoxin contaminated diets compared to broilers fed the control diet or mycotoxin containing diet supplemented with BG-MAX. Broilers fed the mycotoxin contaminated diets had higher AFB1 in the liver compared to broilers fed the control diet. Broilers fed diets supplemented with BG-MAX decreased the AFB1 residue in the liver compared to broilers fed the mycotoxin contaminated diet and were not different from broilers fed the control diet. In conclusion, supplementation with BG-MAX reduced negative effects of mixture of mycotoxins on performance and reduced the carry-over effect of aflatoxin B1 residue in liver.

Key Words: mycotoxins, broilers, aflatoxin metabolism, aflatoxin residue, cytochrome P450

T199 Metabolites produced by the probiotic *Bacillus subtilis* 29784 strengthen intestinal barrier, modulate inflammatory response and gut environment Damien Preveraud¹, Eric Pinloche², Richard Ducatelle, Filip Van Immerseel, Estelle Devillard¹ ¹*Adisseo France SAS*, ²*Adisseo France*

The probiotic strain *Bacillus subtilis* 29784 (Bs29784) produces many metabolites. Niacin (NA), hypoxanthine (HPX) and pantothenate (PTH), have been detected both in vitro (culture media) and in-vivo (digestive tract of poultry). The purpose of this study was to determine the effects of those metabolites on intestinal health using Caco-2 cell models and the impact on the chicken gut microbiota using in-vitro fermentation.

All molecules were tested at 1mM and were compared to non-treated conditions. Cellular assay: cell proliferation was measured for 9 days using PrestoBlueTM. Level of inflammation was evaluated by measuring TER and cytokine release (IL6/IL8) after 2h incubation with the treatment followed by 4h incubation with cytomix (TNF α /IFN- γ /IL-1 β). Microbiota assay: ileal and caecal content (28d chicken) were incubated in anaerobic buffer (5% content/buffer in Hungate tube) for 24h. Gaz pressure, pH, SCFAs, NH₃, lactate were measured and microbiota composition was evaluated using 16S sequencing.

All metabolites produced by Bs29784 increased cell proliferation, up to 73.5%, 59.8% and 50.4% for HPX, PTH and NA, respectively. The drop of TER observed following cytomix stimulation was lower with the addition of both PTH and HPX. HPX treatment led to a significant decrease in IL6 production (-47.3%). In the presence of caecal content, NA increased acetate, HPX increased butyrate while PTH increased acetate, butyrate, and propionate and overall SCFAs. In the presence of ileal content, only PTH increased butyrate. Microbiota composition analyses are in progress.

This in vitro study suggested that the selected metabolites produced by Bs29784 have complementary effects on intestinal epithelial cells by improving cell proliferation, intestinal integrity or decreasing IL6 production. In addition, a shift in the microbial fermentation pattern was observed. Taken together these results strengthen the understanding of the effect of Bs29784 on the host and explain at least partly its positive effects on animal resilience.

Key Words: bacillus subtilis, metabolites, inflammation, barrier function

T200 A220, an all-natural feed additive, reduced *Salmonella* intestinal colonization in broilers and tuned down SPI-1 Type III Secretion System (TTSS) virulence machinery Hongyu Xue¹, Dongping Wang¹, Sara Johnston¹, Billy Hargis², Guillermo Tellez² ¹*Amlan International*, ²*Department of Poultry Science, University of Arkansas*

Antibiotic resistance concerns have driven the need for alternative solutions to antibiotics in reducing *Salmonella* colonization in broiler chickens at the farm level. A220, is an all-natural feed additive that features a proprietary blend of essential oils, fatty acids and an enterosorbent mineral. This formula has been shown to neutralize a variety of key virulence factors of pathogenic bacteria in addition to exerting direct bacteriostatic/bacteriocidal effects. This study was aimed to evaluate *in vitro* and *in vivo* effects of A220 on *Salmonella enterica* sv. *Typhimurium* (ST) infection in broiler chickens.

An *in vitro* digestion model simulating the pH and enzymatic conditions of 3 gastrointestinal segments (crop, proventriculus and intestine) was first used to evaluate the antibacterial effects of A220 on ST. In the *in vivo* trial, one-day old male broiler chicks were randomly allocated to one of three groups (n=30 chickens), i.e., challenged control with non-treated feed and A220 supplemented at 0.25% and 0.5% in feed. All groups were challenged with ST (10⁶ cfu/bird) via oral gavage on d9.

In the *in vitro* trial, A220 significantly reduced total CFU of ST recovered in the proventriculus and intestinal segments compared with control ($P < 0.05$). *In vivo*, A220 0.25 and 0.5% reduced ($P < 0.05$) the total cfu recovered and total prevalence of ST in the ceca. A220 at both doses sig-

nificantly reduced gut permeability after ST challenge as measured by the serum FITC-dextran levels ($P < 0.05$). Further, A220's effects on ST's *Salmonella* pathogenicity island-1 virulence network development were explored via treating ST at subinhibitory concentration (1 mg/mL) of A220 *in vitro*. Compared to the control, A220 downregulated ST *hilA* and *invF* mRNA expression, which further blocked expression of key downstream effectors involved in ST invasion.

Collectively, A220 had the potential to reduce ST colonization in broiler chickens and preserve the functional integrity of the intestinal barrier of chickens during ST challenge. This is at least in part attributed to its anti-virulence properties by interfering with SPI-1 TTSS virulence machinery.

Key Words: *Salmonella* Typhimurium, intestinal colonization, virulence gene expression, *Salmonella* pathogenicity island-1, natural antimicrobial

T201 The effects of in-feed technology (Lumance®) on growth performance and inflammatory gene expression in broiler under dietary challenge Alireza Khadem^{1,2}, DON Ritter², Christos Gougoulas²
¹Ghent University, ²Innovad SA

Poultry are exposed to a number of stress factors, resulting in the activation of the gastrointestinal immune system and the release of pro-inflammatory cytokines. The occurrence and frequency of the inflammatory responses are inversely proportional to animal health and growth performance. An experiment was conducted to evaluate the ability of an in-feed technology (Lumance® Innovad: esterified butyrate, combined with plant extracts, essential oils, and fatty acids) to alleviate the inflammatory responses in broilers. This experiment was carried out with three concentrations of Lumance (0, 1, and 2 Kg/ton) in feed with a high NSP (60% Wheat + 5% rye) without NSPase and coccidiostat (n 8 pens/treatment; n 30 birds/pen). Bodyweight (BW), Feed intake, and FCR were determined on days 14, 28, and 35 and the expression of several jejunal inflammatory genes was determined on day 35. One broiler per pen was randomly chosen on day 35 for the determination of jejunal gene expressions, and the qPCR was performed for iNOS, IL-6, TNF α . Lumance® with 1 and 2 kg/ton increased the BW of broilers by 3 % and 4 % respectively, and broilers fed Lumance® 2 kg/ton were significantly heavier at day 28 and 35 ($P = 0.047$, $P = 0.040$ respectively) compared to the control. Lumance® 2 kg/ton showed significantly lower FCR over 0–5, 2–5 weeks ($P = 0.012$ and $P = 0.038$ respectively). No differences were found between the treatments for FI. The intestinal expression of iNOS and TNF α were significantly lower in Lumance 1 and 2 kg/ton compared to the control ($P = 0.010$ and $P = 0.009$ respectively). The expression of IL-6 was not significantly reduced compared with the control; however, they were numerically lower in the Lumance 1 and 2 groups (1.36 and 0.71) compared to the control group (1.46) ($P = 0.056$). In conclusion, feed with high NSP content can act as a chronic inflammatory trigger, because of the injuries they cause and constant daily exposure to the gut epithelium. Lumance® could reduce the detrimental effect of chronic inflammation thus could counteract the synergistic and additive negative impact of several stress factors, typically seen in the field. Additionally, there is interest in determining if inflammation markers can predict flock health and well-being.

Key Words: Growth, inflammation, Gene expression, NSP, Feed additives

T202 Evaluation of the performance benefits of Balancius in different feeding programs in 56-day old broilers April Levy*, Shelby Corray, Doug Teigte

Peptidoglycans (PGN) are polymers which make up a large component of the cell wall of gram-positive bacteria and are found to a lesser extent in the cell wall of gram-negative bacteria. During the process of cellular division and death, PGN fragments are released into the intestine, where they can interfere with nutrient digestion and absorption. Supplementation of a novel microbial muramidase enzyme (Balancius™, DSM Nutritional Products) has been shown to hydrolyze dead bacterial cell debris contain-

ing PGN in the gastrointestinal tract. The objective of this study was to evaluate the effects of a muramidase enzyme provided in different feeding programs on broiler performance throughout 56-days. A total of 768 day-old Cobb 500 male chicks were randomly allocated to one of four treatment groups, which consisted of T1: a non-supplemented control; T2: muramidase supplementation during the starter and grower phases (d0-28), T3: muramidase supplementation during the starter, grower, and finisher phases (d0-42); and T4: muramidase supplementation in all feed phases (d0-56). Muramidase was provided at 25,000 LSU (F)/kg. Each group consisted of 12 replicate pens with 16 birds per pen. Data were analyzed using the GLM procedures of SAS with significance reported at $P < 0.05$. The PDIF option of SAS was used to compare the broilers fed diets with or without muramidase. For the d0-14, d0-28, and d0-35 periods, muramidase supplementation significantly improved BW, ADG, and FCR compared to T1 ($P < 0.05$). For the d0-42 period, BW, ADG, and FCR was significantly enhanced in T3 and T4 compared to T1 ($P < 0.05$), while T2 was intermediate for all parameters. Similarly, during the d0-56 period, BW and ADG was significantly increased in T3 and T4 compared to T1 ($P < 0.05$), while T2 was intermediate. Though BW and ADG of T3 and T4 were not statistically different, T4 was numerically higher. Feed conversion was improved in T4 compared to T1 and T2 ($P < 0.05$), while T3 was intermediate, and no significant difference was noted between T2 and T1. Overall, the results of this trial suggest that muramidase supplementation may improve performance of broilers raised in small, medium, or large bird programs and termination of supplementation would not be recommended prior to processing.

Key Words: Muramidase, Broiler, Peptidoglycan, Performance, Phase feeding

T203 Effect of a proprietary yeast-based product on weight gain, feed conversion, and carcass yield of Ross 708 broilers Chris Rude*, Muhammed Shameer Abdul Rasheed, Kristy Dorton Devenish Nutrition, LLC

A study was conducted to determine the effects of a proprietary yeast-based product (HT; HY-Tech, Devenish Nutrition, Fairmont, MN) on weight gain, feed conversion and carcass yield of broilers. One day old Ross 708 broiler chicks (n = 900; as hatched) were randomly assigned to 18 pens (50 birds per pen; 1.08 ft²/bird). The 2 treatment groups were: Control (basal diet, no test product) or HT at 1.50, 1.25, 1.00, and 1.00 lb/ton in the starter, grower, finisher, and withdrawal diets, respectively. No coccidiostat program was added to the diets. On day 3, all birds were challenged with a coccidiosis vaccine (CocciVac B-52, Merck Animal Health, Madison, NJ) at 20 times the recommended dose on the feed. Body weight was measured at d 0, 16, 35 and 56. Weight gain and feed conversion were determined from d 0 to 16, d 16 to 35, d 35 to 56, d 0 to 35 and d 0 to 56. On d 58, a subset of male broilers from each treatment were processed for carcass yield. Data was subjected to a one-way ANOVA using the Mixed procedure of SAS 9.4 (2018) with pen as the experimental unit. Live weight of the broilers chosen for yield was used as a covariate for yield measurements. Means were separated by Fisher's protected least significant difference. Differences were considered significant at $P \leq 0.05$. Body weight and weight gain were not significantly affected by treatment. Broilers supplemented with HT had better feed conversion from d 36 to 56 ($P = 0.03$; 2.012 vs 2.060) and d 0 to 56 ($P = 0.0002$; 1.681 vs 1.707). Adjusted feed conversion was also better ($P = 0.06$; 1.684 vs 1.709) when broilers were supplemented with HT. Broilers supplemented with HT had greater post evisceration weight ($P = 0.00522$; 8.393 vs 8.231 lb) and percentage ($P = 0.0048$; 75.12% vs. 73.63), fillet weight ($P = 0.06$; 3.059 vs 2.870 lb) and as a percentage of live weight ($P = 0.03$; 27.44 vs 25.56%) and total boneless weight ($P = 0.06$; 3.596 vs 3.373 lb) and as a percentage of live weight ($P = 0.03$; 32.10 vs. 30.04%). The results show that supplementation of HT improved feed conversion by 2.6 points and carcass yield of broilers. These parameters would have a significant positive

impact on the economics of broiler production, especially for large bird deboning operations.

Key Words: Broiler, Yeast, Feed conversion, Carcass yield

T204 Effect of guanidinoacetic acid on the diet of broilers on productive and economic parameters. Jose Rivera Ulloa^{*1}, Marcela Moreno², Miguel Hernandez², Jairo Carvajal², Judith Ringel³, Vivienne Inhuber³ ¹University of Sao Paulo - Brazil, ²Italcol, ³Alzchem Trostberg GmbH

The objective of this study was to evaluate if the supplementation of guanidinoacetic acid (GAA) either on top or included with an energy matrix in broiler feed, improves or equalizes the productive and economic parameters in broilers, respectively. The study was carried out in an experimental farm located in the department of Santander-Colombia and lasted 34 days. A total of 1088 Ross 308 AP day-old male chickens, with an initial bodyweight of 42.5 ± 1.05 g, were allotted to four treatments, with eight repetitions of 34 chickens per treatment. The experimental design was completely randomized. The treatments were: T1: Control; T2: Negative control (-80 Kcal); T3: T1 + 600 g GAA on top; T4: T2 + 600 g GAA. The trial comprised three phases, in the pre-starter it was up to a consumption of 250 g/bird, the start up to a consumption of 850 g/bird and the fattening was up to day 35 of age. The following production parameters were determined/calculated: feed intake, bodyweight, feed conversion, European Efficiency Index (EEI) and feed cost for each kg of chicken produced. After verification of the assumptions of homogeneity of the variances (Levene's test) and normality of the error (Shapiro-Wilk test), the data were subjected to ANOVA analysis of variance, with a significance level of 5%. When significant differences were found ($P < 0.05$), means were compared using the Tukey test. For data analysis, the statistical program JMP version 15.2 was used. At day 35, the T2 showed 67 g less and 3 FCR points higher than T1 ($p < 0.05$). When we compared the group supplemented with 600 g / ton of GAA considering an energy matrix of 80 kcal with the control group, we found that the GAA group obtained 21 grams more per chicken, 2 points less in feed conversion, a feed cost per kg of meat produced 2% lower ($P < 0.05$) and a return on investment (ROI) of 4.46. When we compared the group supplemented with 600 g / ton of GAA on top with the control group, we found that the GAA group obtained 59 grams more per chicken, 4 points less in feed conversion, a feed cost per kg of meat produced 1.1 % lower ($P < 0.05$) and an ROI of 5.65. In conclusion, the use of GAA both matrixed at 80 kcal and on top, improves the productive and economic efficiency of broilers at 35 days of age.

Key Words: Creatine, Efficiency, Energy matrix, Feed conversion ratio, ROI

T205 Centrifugation Method Affects Digesta Viscosity Michael Barnas^{*1}, Jennifer Timmons², Caleb Nindo² ¹AHPharma, Inc., ²University of Maryland Eastern Shore

Although numerous studies have described the anti-nutritive effects of highly viscous digesta, there is no standard method to measure the viscosity of GI contents. In the field of poultry science, virtually all research published on digesta viscosity involves centrifuging the digesta to extract the supernatant for subsequent analysis using a cone and plate viscometer—a necessary step because the sample volume of digesta is limited in poultry, making mixer viscometry impossible using individual birds. However, centrifugation or dilution with water before measurement can underestimate viscosity and produce variable results. Furthermore, several reports describe centrifugation of digesta at $3,500 \times g$, while others employ $12,000 \times g$ or more. Other studies measure the supernatant viscosity at 25°C, which does not represent the internal body temperature of most animals. Modern rheometers allow the user to better simulate the flow of a substance in its natural environment by dynamically changing numerous parameters such as oscillation frequency and plate temperature. For example, in poultry digesta, gastrointestinal motility data can be utilized

to determine the frequency and amplitude of contractions in various areas of the GI tract. Considering slow waves occur in the duodenum and ileum of broiler chickens at a frequency of 6 cycles/min and the internal body temperature is approximately 40°C, a rheological method was programmed on a Discovery HR-2 Rheometer to mimic this biological process and more accurately predict digesta viscosity *in vitro*. Although the thickness of the whole digesta was dramatically different in the various areas of the GI tract in 21d old broilers, after centrifugation, the viscosity of the supernatant from the upper (3.302 ± 0.44 cP) and lower (3.391 ± 0.48 cP) GI tract were not different ($P > 0.05$). This result questions the utility of existing methods used to quantify digesta viscosity after centrifugation of the sample because the process does not simulate peristalsis *in vivo*. Measuring the viscosity of digesta whole without centrifugation resulted in $3,241 \pm 657$ cP in the duodenum, $6,331 \pm 1738$ cP in the jejunum, and $7,013,500 \pm 137,200$ cP in the ileum of straight-run Cobb-500 broilers at 21d.

Key Words: Viscosity, NSP, Passage Rate, Gut Health, Feed Efficiency

T206 Effect of dietary Moringa oleifera and Linseed in producing eggs enriched with omega 3 fatty acids and lower cholesterol F Sharmin^{*1,2}, M. S. K. Sarker^{1,2}, NR Sarker^{1,2,3} ¹Strengthening of Poultry Research and Development Project, Bangladesh Livestock Research Institute (BLRI), ²Poultry Production Research Division, ³Krishi Gobeshona Foundation, BARC Campus, Farmgate

People are becoming highly aware on food and health over the last decades. A food can be considered functional if it has beneficial effects on human health and decreases the risk of diseases. Eggs are highly favored in the food industry as an excellent source of nutrients and for their valuable functional properties. The objective of the study was to evaluate the feeding effect of *Moringa oleifera* (MOL) and linseed in different combination to the basal diet of Fayoumi laying hens egg quality, yolk fatty-acid composition and the cholesterol content. Ninety-six (96) laying hens (32-48 weeks of age) were considered for this study. The birds were housed in a close, ventilated caged-layer house. They were distributed into four dietary groups having 24 birds in each group with 3 replications consisting 8 birds per replication. The management practices were standard with dietary requirement. There were four dietary treatments (T₁-Control Diet; T₂-0.5% Flaxseed+ *M. oleifera* leaf (MOL) 1%; T₃-1% Flaxseed+ MOL 1%; T₄-1.5% Flaxseed+ MOL 1%). The average egg weight was found to be increased in additives groups. In contrast, total egg cholesterol content in T₃ and T₄ was the lowest with the value of 173.95 and 226.21 mg/100g compared to control group, 283.86 mg/100g. Omega 3 fatty acids in egg (g/100g) of the were increased in T₂ group with the value of 1.867 and 1.55 in T₄ group. In conclusion, *M. oleifera*, and linseed seed combination could be a promising functional feed ingredient to produce value-added egg.

Key Words: Moringa oleifera, Feed additives, Laying hen, Fatty acids, Cholesterol

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

T207 Impact of body-worn sensors on broiler chicken behavior and agonistic interactions Gracie Anderson*, Alexa Johnson, Ahmed Ali *Clemson University*

The importance of evaluating effective poultry welfare measures has increased with public concern. Technology, like body-worn sensors, enables data collection from similar-looking individuals in large groups, but may alter behavior. Our objective was to evaluate the impact of body-worn sensors on broiler behavior and agonistic interactions. In this study, broilers were housed in 8 pens (8.8 birds/m²). At 21 days old, 10 birds/pen were fitted with a harness that held a sensor (HAR), while remaining 10 birds were unharnessed (NON). Instantaneous scan sampling was used to record number of birds performing behaviors (i.e., inactive, locomotory, consummatory) every 10min, while agonistic interactions (i.e., peck, claw) were recorded during nine 20min periods for 21h/day on days 22-26. Counts of birds performing each behavior were calculated as a percent (%) of the total number of birds in each group (HAR or NON). Percent of occurrence of agonistic interactions were identified based on birds involved, resulting in 4 categories: 2 NON birds (N-N), NON aggressor to HAR recipient (N-H), HAR aggressor to NON recipient (H-N), or 2 HAR birds (H-H). Data were analyzed using GLMM in R software (3.3.1). More HAR birds were inactive than NON birds on day 22 (HAR=63.23, NON=57.96; $p<0.05$). HAR birds performed locomotory behavior (i.e., walking: HAR=2.25, NON=3.63) and explored less often (HAR=1.12, NON=3.53) than NON birds on day 22 ($p<0.05$). HAR birds performed comfort behaviors like preening (HAR=2.33, NON=3.36) and stretching (HAR=0.25, NON=0.41) less often than NON birds on day 22 ($p<0.05$). Inactive, locomotory, exploratory, and other comfort behaviors did not differ between treatments on days 23-26, and consummatory behavior was unaffected by treatment on any day ($p>0.05$). Agonistic interactions occurred more often between N-H birds on days 22 (N-H=57.6; H-H=23.52; N-N=13.25; H-N=5.63) and 23 (N-H=36.83; H-H=24.85; N-N=19.7; H-N=18.63) than other categories ($p<0.05$). Agonistic interactions were unaffected by treatment on days 24-26 ($p>0.05$). Broilers adapt to the presence of harnesses and return to normal behavior after 1-2 days, thus a similar acclimation period is required before using body-worn sensors to evaluate broiler activity and welfare without altering behavior.

Key Words: broiler chicken, body-worn sensors, behavior, agonistic interaction, animal welfare

T208 Resource use of free-range laying hens in response to a mobile robot Alexa Johnson*, Gracie Anderson, Ahmed Ali *Clemson University*

Advances in robotics can be applied to livestock systems to promote resource use. However, limited research is available for laying hens in free range systems. The objective of this study was to 1) test the effectiveness of a robot in restricting range access and 2) evaluate resource use of hens within the house. A total of 128 Brown Leghorn hens were housed in 8 free-range pens (16 birds/pen) with the door to the range area open for 18 hours/day (6:30-20:30). Four control (CON) pens had unrestricted access to the range when it was available. The remaining 4 pens contained a robot (ROB) that moved at a steady pace in front of the range door inside the house for the first 5 hours of range access, preventing unrestricted access to the range. The robot was placed when the hens were 40 weeks old. An acclimation period of 24 hours to the robot occurred before data collection. Instantaneous scan sampling was used to record the number of birds on litter, perches, nest boxes, and range every 10 minutes from 6:30-11:30 every day for 14 days. Data were analyzed in R software (v3.3.1) using GLMM and Tukey's post-hoc test. On the first day of observation, 0% of hens in ROB pens were found in the range compared to 62.5% of hens in CON pens ($p\leq0.05$). Similarly, a higher percentage of birds in ROB pens (67.8%) used perches than birds in CON pens (6.3%; $p\leq0.05$). By the sixth day of observation, the percentage of birds on perches was similar across treatments ($p>0.10$). On the first day of observation, hens in ROB pens were found in the litter less often compared to CON birds (6.25% vs. 25%; $p\leq0.05$). On the third day of observation, more birds in ROB pens (53%) were found on the litter than CON pens (18.75%; $p\leq0.05$). On average, 25% of hens in ROB pens were observed inside nest boxes daily during morning hours compared to 10% of CON birds ($p\leq0.05$). Our results suggest the robot succeeded in preventing access to the range. Disturbances in perch and litter use in response to robot introduction were diminished gradually and disappeared by the sixth day. The robot constrained hens to use the resources inside the house, such as nest boxes. Further research is needed to examine the robot's impact on egg production and egg laying location.

Key Words: layer hens, behavior, free-range, robotics, resource use

POSTER SESSIONS

Teaching, Pedagogy, Extension

P209 Quantification of non-commercial poultry shipments into Kentucky Jennifer Hall¹, Caroline Meek¹, Anthony Pescatore^{*2} *¹Kentucky Poultry Federation, ²University of Kentucky*

There has been an increase in the number of people interested in raising small flocks of poultry. Anecdotal information indicates that this increased interest has resulted in the number of non-commercial birds that are being shipped into Kentucky. This information on shipment of birds can be from three sources: a certified veterinary inspection form, National Poultry Improvement Plan (NPIP) VS 9-3 form, or a NPIP VS 9-3I form. Since there is no central database on shipments and much of the information is transferred as paper copies, it is difficult to quantify the number of birds shipped. This type of information is desirable in order to develop Animal Health Emergency plans and outreach programs.

In order to get this information, an effort was undertaken to summarize VS 9-3 and VS 9-3I forms for shipments into Kentucky from January to August of 2021. During this period, there were 5,478 poultry shipped

directly to 3,890 individual premises with a total 306,419 birds. Chickens accounted for 61 percent of the shipments, pheasants for 15 percent and 7 percent each for ducks and quail. Shipments came from 39 states with Iowa, Ohio, Missouri and California being the most frequent states.

Two-thirds non-commercial birds were shipped into Kentucky through large chain farm stores and local farm stores. The farm stores received a total of 693,176 birds during this period with chickens made up 74 percent of the total birds shipped. The resale of birds through these outlets does not allow for identifying the number of premises that received poultry.

A review of shipments into Kentucky indicated that 999,595 non-commercial birds were shipped into the state. The distribution of two-thirds of these birds in Kentucky could not be determined due to the lack of records of individual sales at feed stores. This information emphasizes the impor-

tance of outreach programs and the inclusion of non- commercial poultry when developing emergency health plans.

Key Words: Noncommercial, interstate shipments

Physiology, Endocrinology and Reproduction: Broilers

P210 Antibiotic supplementation improves jejunal tissue damage during a subclinical necrotic enteritis challenge in broilers Shailes Bhattarai^{GS}, Laura E. Ellestad *University of Georgia*

Antibiotics have been used in poultry feed for several decades to promote intestinal health and enhance production efficiency. However, conflicting findings on how antibiotics impact bird intestinal health during *Clostridium perfringens* (CP)-induced subclinical necrotic enteritis (NE) in broilers still exists. This study evaluated the role of bacitracin methylene disalicylate (BMD) on intestinal physiology during a subclinical NE challenge in broilers. Day-old male Ross broilers were grouped into one of three treatments (n=5 pens/treatment): unchallenged without BMD supplementation, challenged without BMD supplementation, and challenged with BMD supplementation. Birds in challenged groups were orally gavaged with coccidia oocytes on D14, followed by oral doses of CP from D19-D21. At 24h, 48h, 96h, and 192h post-challenge, one bird per pen was orally gavaged with fluorescein isothiocyanate dextran (FITC-d) and blood was collected 2h later. Jejunal mucosa samples were collected from one additional bird per pen at the same time points. Levels of FITC-d in plasma were measured by fluorometry, and mRNA expression for jejunal tight junction (TJ) and inflammatory genes were measured by RT-qPCR. Data were analyzed by two-way ANOVA followed by Fishers Least Significant Difference when ANOVA indicated significance ($p < 0.1$). Plasma levels of FITC-d did not differ significantly. The mRNA expression of pro-inflammatory and anti-inflammatory cytokines, *IL-1B* and *IL-10*, respectively, was downregulated by NE, and BMD supplementation reversed these effects ($p < 0.05$). Similarly, mRNA expression of *IFN-α* was downregulated by NE and lowest at 24h post-challenge ($p < 0.05$), but in this case, BMD supplementation further reduced its levels. The mRNA expression of TJ genes *CLDN-1*, *OCLN*, *TJP-1*, and *MUC-2* was downregulated by NE, and BMD supplementation reversed this effect for *CLDN-1*, *TJP-1*, and *MUC-2* ($p < 0.05$). The mRNA expression of *CLDN-1* was highest at 24h post-challenge ($p < 0.05$). These findings provides evidence that BMD minimizes jejunal inflammation by downregulating key inflammatory cytokines and improves jejunal integrity by regulating TJ genes, thereby suggesting a potential protective mechanism for BMD in jejunal tissue damage during a subclinical NE challenge in broilers.

Key Words: anti-inflammatory, bacitracin methylene disalicylate, pro-inflammatory, tight junction

P211 Investigating the effect of genetic selection on growth performance and intestinal density in three random bred broiler populations Maricela Maqueda^{GS}, Kirsten Shafer, Travis Tabler, Sara Orłowski *Department of Poultry Science, University of Arkansas*

Genetic selection of the commercial broiler has helped improve growth performance, feed efficiency and processing yields. Utilizing random bred broiler populations representing snapshots in time of the genetic progress of the commercial broiler can help determine what has been altered in the broiler through genetic selection for efficiency traits. Three random bred broiler lines, representative of commercially available genetic stock from the 1950s (ACRB), 1990s (RAN), and 2015 (MRB), were evaluated for growth performance, relative organ weights, and intestinal density. A total of 120 birds per line were fed *ad libitum* for eight weeks. Six birds per line (3 males and 3 females) were harvested at 0, 1, 2, 3, 4 and 8 weeks. At each sampling body weight, yolk sac weight, liver weight, and small intestine segment weights and lengths were recorded. Means were separated using Tukey HSD (JMP Pro 16.0) with significance at $P < 0.05$. Growth rate

and body weights differ greatly among the lines, so organ weights were expressed as a percentage relative to live weight and intestinal density was calculated as a ratio of weight to length to facilitate comparisons. Week 0 weights were heaviest in the MRB (53.11 ± 1.47) as expected. At every sample time, the ACRBs exhibited lower body weights. At 8 weeks, the MRB line had the highest body weight (3463.15 ± 184.08), the ACRB the lowest (787.75 ± 61.92) and the RAN an intermediate (2372.78 ± 201.41). Yolk sac percentages were highest in the MRB, and lowest in the RAN line at week 0. The RAN line had the highest liver percentage at week 1. Duodenal and ileal percentages did not differ between the MRB and RAN lines. Intestinal density did not differ between lines at hatch, however, by week 8, the MRB line had the greatest intestinal density in the duodenum while the MRB and RAN lines did not differ for the ileum or jejunum at that age. This insight suggests that genetic selection has increased growth rate and feed efficiency by promoting the progression of intestinal density in the broiler.

Key Words: digestive tract, selection, intestinal density, growth performance, small intestine

P212 Effect of cyclic heat stress on the hypothalamic oxygen homeostasis and inflammatory state in different broiler populations and their ancestor Giorgio Brugaletta^{1,2}, Elizabeth Greene², Travis Tabler², Sara Orłowski², Federico Sirri¹, Sami Dridi² ¹*Department of Agricultural and Food Sciences, Alma Mater Studiorum – University of Bologna*, ²*Center of Excellence for Poultry Science, University of Arkansas*

Heat stress (HS) imperils the poultry production sustainability worldwide. Furthermore, hyper-selected chickens are less able to endure HS than their predecessors due to higher metabolic rates. Performance losses caused by HS are associated with hypoxia and decline in blood supplies to internal organs, including the hypothalamus. The hypothalamus is a vital control center of FI and energy homeostasis, mainly via its neuropeptides. However, intriguing pathways involving hypoxia inducible factor (HIF) have been shown to play a pivotal role in hypothalamic FI regulation. The present study aims, therefore, to determine the effects of chronic cyclic HS on the hypothalamic oxygen/hypoxia-associated markers in the chicken ancestor junglefowl (JF) and three broiler lines from diverse phases of genetic selection (i.e., the slow growing ACRB, the moderate growing 95RN, and the fast growing MRB). Birds were subjected to either thermoneutrality (TN, 25 °C) or cyclic HS (36 °C, 0900-1800 h) from day 29 to 56. Hypothalamic mRNA expression data were analyzed via two-way ANOVA with temperature and line as the main factors. HS did not affect the expression of HIF system in any studied population, however it upregulated the expression of NF-κB gene in JF ($p < 0.05$). NF-κB1 expression was higher in JF than MRB under TN ($p < 0.05$). The expression of tumor necrosis factor-α (TNFα) was greater in 95RB than MRB under both TN and HS ($p = 0.06$). The expression of AMP-activated protein kinases (AMPK) and mechanistic target of rapamycin (mTOR) was unaffected while that of ribosomal protein S6 kinase (RS6K) was higher in 95RB and MRB than ACRB regardless of the temperature. This is the first attempt to unveil the effects of HS on the hypothalamic oxygen homeostasis and inflammatory state of unselected chicken and differently performing broilers.

Key Words: Broiler chicken, Ancestor, Heat stress, Hypothalamic oxygen homeostasis, Hypothalamic inflammation

P213 A Thermal Imaging Method for Evaluating Broiler Growth Parameters Yangyang Guo¹, Ade Oladeinde^{1,2}, Romdhane Rekaya³, Ahmed Ghareeb¹, James Foutz¹, Gregory Zock¹, Ramesh Bist¹, Samuel Aggrey¹, Lilong Chai¹ ¹*Department of Poultry Science, University of Georgia*, ²*U.S. National Poultry Research Center; USDA ARS*, ³*Department of Animal and Dairy Science, University of Georgia*

The rapid growth of broilers is associated with welfare concerns such as lameness or weak legs that could lead to the suffering of chickens from some behavior restrictions, physical discomforts, and impinge fundamental freedoms. Those welfare concerns have triggered the attention from the general public and the primary restaurants or grocers to improve broiler well-being, and welfare assessments. However, there is no automated method that can evaluate body and leg growth simultaneously. Such methodology could have gait score implication. The objectives of this study were to collect images of growth parameters with age. In this study, 120 Cobb-500 broiler chickens were raised (day 1- 49) and monitored in a research broiler house (i.e., 6 pens each with 20 birds). A thermal camera was used to collect images of broilers and their legs as thermal images have higher resolution in identifying legs' (e.g., joints, drum sticks, and shanks) as compare to conventional 2D images. A machine vision-based model (MVB) was developed (based on 600 legs thermal images) and

tested in detecting leg parts and quantifying legs distance and changes with ages (e.g., d14, d21, d28, d35, and d42). Broiler legs' thermal images were defined and grouped as normal legs distance (NLD: averaged legs distance was 5.44 ± 0.28 to 9.04 ± 0.34 cm from d14 to d42) and large legs distance (LLD: averaged legs distance was 6.76 ± 0.38 to 10.94 ± 0.51 cm from d14 to d42) for training the MVB model to analyze thermal images automatically. The legs distance and body weight of broilers at different ages were compared with one-way ANOVA in MATLAB 2019. Results show that birds' body weight and their legs distance were linearly correlated ($Y = 549.8 \times \text{x-leg distance}$ $R^2 = 0.9065$). For grouped NLD images, birds had significant increase of body weight and leg distance from d14 to d42 ($P < 0.05$). However, grouped LLD images were found with no difference in legs distance on d35 and d42 (i.e., 10.41 cm on d35 vs 10.94 cm on d42; $P = 0.197$). The possible reason was that legs distance has been maximally formed on d35 already. In this study, we developed the reference legs distance for birds at different ages or body weight. The relationship between body weight and leg distance can be explored further in gait score analysis.

Key Words: Poultry production, machine vision, leg parameters, thermal imaging, animal welfare

Physiology, Endocrinology and Reproduction: Laying Hens

P214 Effect of mixed *Eimeria* challenge on skeletal health of Hy-Line W-36 pullets Milan Sharma^{*GS}, Dima White, Yuguo Tompkins, Guanchen Liu, Woo Kim *Department of Poultry Science, University of Georgia*

An experiment was conducted to determine the effect of mixed *Eimeria* challenge on skeletal health of Hy-Line W-36 pullets. A total of 540, 16-d old pullets were randomly allocated into five treatment groups, including a non-challenged control. Mixed *Eimeria* species solution containing 50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* oocysts per mL were prepared and challenged to one group as a high-dose treatment. The 2-fold serial dilution was done to prepare the medium-high (25,000 *E. maxima*; 25,000 *E. tenella*; 125,000 *E. acervulina*), the medium-low (12,500 *E. maxima*; 12,500 *E. tenella*; 62,500 *E. acervulina*), and the low (6,250 *E. maxima*; 6,250 *E. tenella*; 31,250 *E. acervulina*) dose treatments and challenged to three corresponding groups, respectively. Dual Energy X-ray Densitometry (DEXA) was used to measure the bone mineral density (BMD) and bone mineral content (BMC) of pullets on 6 and 28 d post-infection (DPI). The rate of femur bone growth was measured from 0-14 DPI and 14-28 DPI using Calcein injection. The properties of the femur, including the cortical and trabecular bones,

were analyzed at the metaphysis region of the femur, using the Skyscan X-ray microtomography on 6, 14, and 28 DPI. Data were analyzed using one-way ANOVA, and orthogonal polynomial contrasts were used to determine the effect of increasing oocyst doses on measured responses. A significant level was set at $P \leq 0.05$, and means were separated using Tukey HSD. The result indicated a significant linear reduction in overall BMD and BMC of the body ($P < 0.05$) using DEXA. The rate of bone growth decreased linearly with an increase in the challenged dose ($P < 0.05$) from 0-14 DPI. Using the X-ray microtomography, cortical and total BMD, BMC, bone volume (BV), bone volume as a fraction of tissue volume (BV/TV) of femur decreased both linearly ($P < 0.05$). In contrast, the total number of pores increased linearly with an increase in challenge dosages on 6 and 14 DPI. Trabecular BV, BV/TV, trabecular number, and trabecular thickness decreased linearly with an increase in the challenge dosages ($P < 0.05$) on 6 DPI. The above results indicated that the mixed *Eimeria* challenge linearly reduces the quality of skeletal health with an increase in the concentration of *Eimeria* oocysts.

Key Words: Pullets, *Eimeria*, DEXA, Skeletal health, X-ray microtomography

Physiology, Endocrinology and Reproduction: Layer or Broiler Breeders

P215 Primary and secondary immune responses in Light-brown Leghorn pullets vaccinated with autogenous *Salmonella* vaccines Chrysta Beck^{*GS}, Jossie Santamaria, Marites Sales, Gisela Erf *University of Arkansas System, Division of Agriculture, Department of Poultry Science*

Poultry *Salmonella* vaccines were developed to manage foodborne salmonellosis, but few studies examined the quality of the immune responses in pullets. The objective of this study was to analyze the primary and secondary, local cellular and humoral, immune responses of Light-brown Leghorn pullets to two autogenous *Salmonella* vaccines (SV1 or SV2). For this, one group of pullets (14-16 wks) received a primary immunization by intradermal (i.d.) injection of vaccines into the pulp of growing feathers (GF); another group received a primary immunization by subcutaneous (s.c.) injection and a second by i.d. GF injection four weeks later. For primary and secondary GF-injections, 20-24 GF per pullet were injected with 10 μ L of SV1 or SV2 each (4 birds/treatment). To examine local cellular

responses, injected GFs were collected before (0 h) and at 6, 24, 48 and 72 h p.i. for immunofluorescent staining and leukocyte population analysis by flow cytometry. To examine the humoral responses, blood was collected before (0 d) and at 3, 5, 7, 10, 14, 21 and 28 d post-primary and -secondary vaccinations. SE-specific plasma IgM, IgG and IgA levels were quantified by ELISA. Data were analyzed by two-way ANOVA. After the primary GF injection of SV1 and SV2, pulp leukocyte infiltration reached highest levels (% pulp cells) by 6 h and declined to near pre-injection by 72 h ($P < 0.001$). However, in immunized pullets, the rapid leukocyte infiltration in response to SV1 and SV2 injection continued to increase from 6 to 72 h ($P < 0.001$). In both the primary and secondary local GF responses, heterophils predominated, followed by macrophages, with minimal participation of T and B lymphocytes. Regarding the SE-specific humoral response, IgM levels were greatly elevated from 5 to 14 d during the primary and secondary response. Elevated IgA levels also were observed during the

primary and secondary response from 5 to 14 d but at 20 times lower levels than IgM. On the other hand, IgG levels increased steadily to maximal levels by 28 d during the primary response and were elevated earlier (7 d) and at much higher levels during the secondary response. These data show that SV1 and SV2 vaccines induced both local cellular and systemic IgG antibody responses with primary and memory phenotype.

Key Words: autogenous *Salmonella* vaccine, pullets, immune response, leukocytes, immunoglobulin

P216 Effect of *Histomonas meleagridis* infection during the onset of lay on mortality and egg production in broiler breeders. Sophie Chance^{UG}, Catherine Fudge, Katherine Cupo, Benjamin Alig, Robert Beckstead, Chongxiao (Sean) Chen *North Carolina State University*

Histomonas meleagridis is a protozoan that causes histomoniasis (black-head disease) in turkeys. Chickens have shown better resistance to the disease than turkeys. However, they may experience an acute infection from which they appear to recover quickly. Previously, we found that early infection (25-day-old) with *H. meleagridis* has limited effects on egg production and quality in broiler breeders. However, broiler breeders are more susceptible to disease during the onset of lay since they are physiologically stressed due to producing eggs. The purpose of this research was to study the impact of *H. meleagridis* infection during the onset of

lay on mortality and egg production in broiler breeders. 24 twenty-three-week-old breeder hens and 6 roosters (Ross 708SF) were randomly placed in six pens with four hens and one rooster in each pen. Three pens were cloacally infected with 100,000 cells of *H. meleagridis* per bird one week after the first egg was laid, whereas the other three pens were controls. The trial was terminated two weeks post infection. The egg production was recorded daily. Since the hens did not start laying simultaneously, the egg production was calculated as overall hen-day-production (HDP) and hen-day-production after they started laying. The liver and ceca of all birds were scored for histomoniasis at termination. The data were analyzed using student *t* test with SAS 9.4 ($P < 0.05$). There were no mortalities during this trial. The hens infected with *H. meleagridis* received average cecal scores of 2.25, while all the control pens received cecal scores of zero. Furthermore, no lesions were found in the livers from either treatment. There is no significant difference in overall HDP ($P = 0.7256$) and HDP after laying ($P = 0.4066$) between infected and non-infected control. These results indicated that *H. meleagridis* infection during the onset of lay did not significantly affect egg production in broiler breeders. However, a large-scale and long-term trial needs to be performed to confirm the current findings.

Key Words: *Histomonas meleagridis*, Histomoniasis, Broiler Breeder, Egg Production

Physiology, Endocrinology and Reproduction: Turkeys

P217 The detection of antibody responses to histomoniasis in turkeys. Sophie Chance^{*1}, Paige Rohlf², Christina Sigmon¹, Robert Beckstead¹, Chongxiao (Sean) Chen¹ *¹North Carolina State University, ²Aviagen Turkey*

Histomoniasis is a protozoan disease caused by *Histomonas meleagridis* most commonly found in turkeys. Infected turkeys display clinical signs of drooping head, depression, and sulfur-colored droppings. The ceca become inflamed, and the liver becomes necrotic, leading to nearly 100% mortality. Nitarsone was the only treatment to Blackhead disease up until its deregulation in 2016. Currently there are no approved vaccines or treatments to histomoniasis resulting in persistent and detrimental outbreaks. Therefore, alternative pathways to histomoniasis treatment such as genetic resistance must be considered. This research focused on the immune response of the few remaining survivors of histomoniasis challenged flocks. 1,647 female poults were placed in two rooms where they were raised to 80 days of age. All the birds were cloacally infected with 1mL of 100,000 histomonads/bird on day 18, 36, and 45. At the end of the trial remaining

birds were necropsied. Cecal and liver lesions caused by *H. meleagridis* infection were scored on a 0-4 scale with 4 being the most severe. The data are present by the number of birds due to the purpose of study is looking for the histomoniasis-resistant individual. Statistical analysis did not apply. The results showed 123 birds survived from the challenges. 62 birds displayed no clinical signs of histomoniasis and 12 others had mild or recovering signs. From these birds, cecal samples were taken and their blood plasma was further analyzed using Windshich and Hess's ELISA with bound *H. meleagridis* cells. The ELISA results showed that 42 of the 74 birds had an antibody response and recovered. The cecal samples also unveiled that 17 of the 62 birds absent of clinical signs were carrying *H. meleagridis*. These findings lead to the conclusion that turkeys are capable of resistance to histomoniasis and give hope to a genetic possibility in reducing outbreaks. Further investigation needs to be done on specific SNPs using a Genome-Wide Associations Study (GWAS) with blackhead disease resistance.

Key Words: Histomoniasis, *Histomonas meleagridis*, ELISA, Antibody, Turkey

Processing and Products

P218 The biofilm forming ability of different *Salmonella* serotypes on stainless steel Hudson Thames^{*GS}, Diksha Pokhrel, Anuraj Sukumaran *Mississippi State University*

Biofilm formation by *Salmonella* on processing surfaces is an emerging food safety concern for poultry processing. Common sanitizing practices may not fully remove *Salmonella* biofilms, allowing persistent shedding of viable cells, thus increasing the risk for broiler meat contamination. The objective of this study was to characterize the biofilm forming ability of different *Salmonella* strains on stainless steel using a crystal violet assay in conjunction with quantification. Five *Salmonella* strains were used in this study: *S. Typhimurium* (ATCC 14028), *S. Enteritidis* (ATCC 4931), *S. Reading* (turkey outbreak strain 0330 and reference strain 0326), and *S. Kentucky* (broiler meat isolate). Six stainless steel coupons were immersed individually in wells of a 24-well culture plate containing 1 mL of

Salmonella culture (6 log CFU/mL) in TSB or 1mL of TSB as a control. The plates were incubated aerobically for 48hrs at 37°C. Biofilm density on the coupons was determined using crystal violet by measuring the optical density (OD₆₀₀) using a spectrophotometer (3 coupons/strain). Simultaneously, three coupons were vortexed in TSB and plated on tryptic soy agar plates to quantify biofilm cells. Data were analyzed by the GLIMMIX procedure of SAS 9.4 at a significance level of 0.05. There were no differences between the optical densities of the five *Salmonella* strains and the control ($P = 0.184$). However, all 5 strains displayed densities above the optical density cutoff (O.D.c) of 0.065: *S. Typhimurium* (0.081), *S. Enteritidis* (0.079), *S. Reading* 0330 (0.078), *S. Reading* (0.076), and *S. Kentucky* (0.094). Quantification of biofilm-attached cells indicated that all *Salmonella* strains formed biofilms on the coupons. Average log CFU/coupon populations of *S. Typhimurium*, *S. Enteritidis*, *S. Reading*

0330, *S. Reading* 0326, and *S. Kentucky* were 6.4, 5.9, 5.9, 6.2, and 5.9, respectively, as compared to no biofilm formation (0 log CFU/coupon) in the controls ($P < 0.001$). However, there were no differences between the strains ($P = 0.144$). These results suggest that quantification may be more accurate when characterizing biofilms, rather than optical density, as OD₆₀₀ values indicated all *Salmonella* strains were weak biofilm formers, despite high concentrations of cells being recovered.

Key Words: *Salmonella*, Biofilm, Processing, coupon

P219 Relative resistance of *Salmonella* serotypes (Typhimurium, Infantis and Reading) to peroxyacetic acid on chicken wings Sasikala Vaddu^{*GS}, Jinquan Wang, Thiago Belem, Gaganpreet Sidhu, Cortney Leone, Manpreet Singh, Harshavardhan Thippareddi *Department of Poultry Science, University of Georgia*

Peroxyacetic acid (PAA) is widely used during poultry processing as an antimicrobial. Recent Salmonellosis outbreaks caused by *Salmonella* Infantis from chicken products and *Salmonella* Reading in turkey products have raised concerns about their enhanced resistance to commonly used antimicrobial interventions such as PAA compared to *Salmonella* Typhimurium in poultry processing. The objective of this research was to evaluate the efficacy of PAA on the three serotypes of *Salmonella* (Typhimurium, Infantis and Reading) and its effect on moisture gain, product color and decomposition of PAA at different pH levels. Fresh chicken wings (0.45 kg) were inoculated with a cocktail (ca. 6 log CFU/mL) of nalidixic acid resistant *Salmonella* Typhimurium (ST^{NA}), rifampicin resistant *Salmonella* Infantis (SI^{Rif}) and kanamycin resistant *Salmonella* Reading (SR^{Kan}). Inoculated chicken wings were immersed in PAA solutions (100 or 500 ppm) adjusted to either pH 8.5 or left unadjusted for either 10 s or 60 min. Treated chicken wings were rinsed in chilled buffered peptone water (100 mL), serially diluted in peptone water supplemented with either 200 ppm Nalidixic acid, rifampicin or kanamycin for enumeration of ST^{NA}, SI^{Rif} and SR^{Kan} respectively and plated on APC PetrifimTM. Immersion of chicken wings in 500 ppm PAA for 60 min resulted in greater microbial reductions of ST^{NA}, SI^{Rif}, SR^{Kan} of about 2 log CFU/mL each. Regardless of concentration and pH of PAA, increased exposure time (60 min vs 10 s) resulted in significant reductions ($P < 0.05$) of ST^{NA}, SI^{Rif}, SR^{Kan}. At all experimental conditions (PAA conc, pH and exposure times) *S. Typhimurium* was more resistant to PAA solutions than *S. Infantis* and *S. Reading* ($P < 0.05$). Rapid decomposition rates of PAA (100 and 500 ppm) at pH 8.5 than at unadjusted pH was observed ($P < 0.05$). The immersion treatments had no effect on moisture gain except for the immersion in pH unadjusted PAA concentrations (100 and 500ppm) for 60 min ($P < 0.05$). Product color (lightness, L*) was not affected by the PAA concentration, exposure time or the pH levels. Monitoring the relative resistance of different *Salmonella* serovars to antimicrobials used in poultry processing such as PAA is indispensable to reduce the foodborne outbreaks and ensure poultry processors.

Key Words: Peroxy acetic acid, *Salmonella*, serotypes, chicken wings, decomposition

P220 Rapid quantification in *Salmonella* spp. in artificially and naturally contaminated ground turkey using a real-time PCR assay Micah Black^{*IGS}, Bet Wu Alvarado¹, Laura Garner¹, Charles Herron¹, Aftab Siddique¹, Adam Joelsson², Vikrant Dutta³, Heath LaFevers³, John Mills³, Amit Morey¹ ¹Auburn University, ²Invisible Sentinel, ³bioMérieux, Inc.

Introduction: *Salmonella* spp. quantification using the USDA/FSIS MLG 4.10 Most Probable Number (MPN) method takes 4-7 days. Rapid real-time PCR (RT-PCR) based methods can provide results in less than 4 hours enabling poultry processors to make food safety decisions sooner. The objective of the research was to evaluate GENE UPâ Quant *Salmonella*, a real-time PCR assay targeting pan-*Salmonella* genetic signatures to quantify *Salmonella* in artificially and naturally contaminated ground turkey samples.

Materials and Methods: Artificially contaminated (with *Salmonella* Typhimurium), raw ground turkey meat obtained from a commercial processor was divided into 24 test portions of 125 g and homogenized with buffered peptone water (1:6). The homogenized samples were randomly assigned (n=6) to four treatments: non-inoculated control, low inoculum (10 cfu/g), medium inoculum (100 cfu/g), and high inoculum (1,000 cfu/g). Naturally contaminated, commercially processed raw ground turkey samples (n=20) were aliquoted into 125 g test portions and homogenized with BPW (1:6). The homogenates (40 mL) from each artificial and naturally contaminated samples were analyzed using the GENE UPâ Quant *Salmonella* assay. The quantification results were compared using a modified MPN method (333.3 mL) combined with GENE UPâ SLM2 analysis. A t-test ($p < 0.05$) was conducted to determine significant differences between the predicted and confirmed *Salmonella* quantification values for the artificially and naturally contaminated samples.

Results: *Salmonella* quantification with GENE UPâ Quant *Salmonella* was within 1 log CFU and 97% comparable to the MPN method ($p > 0.05$). Similarly, the difference between the predicted and confirmed *Salmonella* levels in naturally contaminated samples was less than 1 log CFU/g and the methods were not significantly different ($p > 0.05$). Results indicate that rapid RT-PCR methods (4 h) such as the GENE UPâ Quant *Salmonella* can be used for enrichment-free quantification of *Salmonella* in raw ground turkey samples.

Key Words: *Salmonella* quantification, Most probable number, Real-time PCR, Ground turkey meat

P221 Rapid Detection of Woody Breast Myopathy in Live Birds, Pre- and Post-Chill WOGs, and Fillets Using Radio-frequency Waves and Machine Learning Algorithms Aftab Siddique^{*IGS}, Charles Herron¹, Laura Garner¹, Amit Morey¹, Ryan Freeman² ¹Auburn University, ²Compass Technology Group, LLC

Rapid and non-invasive technologies to detect woody breast in live broilers and at various processing steps is of significant interest to the global poultry industry. We investigated the application of radio-frequency waves (2-18 GHz) to detect woody breast in live broilers, and at various processing steps.

Ross 708 broilers (48 days old; n = 108) were analyzed using a portable radio-wave transmitter device (2-18 GHz) (<1 sec) on live birds, pre-chilled WOG's, post-chilled WOG's (4°C), and deboned fillets. Amplitude and phase of each frequency were gathered in two different positions (waves perpendicular to muscle fibers and waves parallel to muscle fibers) (3200 data points x 2 positions). Deboned fillets were hand-palpated and scored for the severity of woody breast myopathy (Normal = 0; Moderate = 1; and Severe = 2) and used as a reference to train the supervised learning algorithm (K-Nearest Neighbor) for classification of live birds, pre- and post-chilled WOG's, and fillets. False discovery rate (FDR) analysis was conducted to eliminate false-positive results and avoid data complexity of the model.

Radio frequency waves were able to detect woody breast in live birds and at different processing steps with varying levels of accuracy. Models were able to predict normal and severe breast meat in live birds at 75-76% accuracy rate. Differences in pre- and post-chill WOGs classification accuracies indicate an effect of water on the radio-frequency method. Classification accuracy of fillets was 82.60 % for Normal, 50.30%-Moderate, 66.70%-Severe woody breast. Woody breast frequency signatures contributing to the classification were different for live birds compared to the WOGs and fillets.

Radio-frequency waves can be used as a rapid method to detect woody breast in live birds, and at different processing steps. Machine learning algorithms can be effectively used to analyze enormous amounts of data

and classify woody breast. Future research should focus on using different machine learning models and improve the accuracy of woody breast detection.

Key Words: Supervised learning Algorithms, K-Nearest Neighbor, Myopathic conditions, Radio-wave frequencies

P222 Efficacy of room-temperature plasma to eliminate foodborne pathogens in ground and ready-to-eat meat Bet Wu^{*1GS}, Emefa Monu², Shijie Qin¹, Amit Morey¹ ¹*Department of Poultry Science Building, Auburn University*, ²*Ontario Ministry of Agriculture, Food and Rural Affairs*

Consumer trends indicate a higher demand for low thermally processed foods without synthetic antimicrobials. Room-temperature plasma consists of ions that can interact with microbial cell-wall, penetrate inside the organism, and kill it. The project investigated the application of room-temperature plasma to eliminate *E. coli* in ground beef and *Listeria innocua* in fully cooked, ready-to-eat ham.

Listeria innocua and *Escherichia coli* k-12 (49595) were grown in Brain Heart Infusion broth (BHI) and trypticase soy broth (TSB), respectively. After incubation time (37 °C/24 h), cultures were centrifuged at 5000 g for 10 and 20 min, respectively. The supernatant was discarded, each pallet was resuspended in 1:1 ratio of 0.1 % peptone water, vortexed and centrifuged again, separately. A final concentration of 10⁷ CFU/mL was used for inoculating the foods. Partially frozen beef was cut using sterile knife (2 x 2 sq. cm) while circular ham slices (1 mm thickness, area 1.6 cm²) were cut using a sterile punching tool. An inoculum (0.05 mL) of *E. coli* K-12 and *L. innocua* were spread evenly on the ham and placed in biosafety cabinet for 1 h to allow for bacterial attachment. Samples were located 10 mm away from cold plasma (He⁺) plume with an exposure time of 0, 5, 10 and 15 min, using a electrical model ranged of 6.8 to 7.04 kV (27mA) with a gate drive signal of 6.5 kHz frequency with a width of 1.0 ms. Microbiological sampling using swab contact and spread plate method was conducted to determine colony forming unit counts (CFU/g). This experiment was performed in triplicate, difference between treatments were analysis using ANOVA (p<0.05).

Room-temperature plasma eliminated 1.75 log CFU/g of *E. coli* after 5 min of exposure (p<0.05) but extended exposure times of 10 and 15 min did not yield significantly lower populations (p>0.05). Reductions of *L. innocua* were 1.02, 1.35 and 1.75 log CFU/g after 5, 10 and 15 min, respectively, were significant (p<0.05).

Room temperature plasma has exhibited the potential to eliminate foodborne pathogens in ground and ready-to-eat products.

Key Words: Cold plasma, Exposure time, Food quality, Foodborne reduction, Food safety

P223 Replacing “First-In First-Out” model with “First-Expire First-Out” to predict shelf-life of raw chicken in each box on a pallet under simulated cold-chain disruption Charles Herron^{*GS}, Telah Black, Aftab Siddique, Laura Garner, Bet Wu, Jewel Johnson, Moni Fadamiro, Amit Morey *Auburn University*

The food supply chain uses the “first-in first-out” (FIFO) model where a pallet is sequenced for placing on shelves as per the receiving data without considering the effects of cold-chain disruption on food safety and shelf-life. Objectives of the study were: 1. Evaluate the effect of cyclic TA of a pallet of raw chicken on its shelf-life; 2. Predict the risk of each box on the pallet crossing 4°C due to TA (5 trials); 3. Predict the “first-expire first-out” for each box on the pallet (2 trials). A commercially produced pallet of raw tray-packed, boneless, skin-less breast fillets was procured from a processor. The pallet consisted of 20 total boxes arranged in 5 boxes per layer (total 4 layers) x 24 tray packs of fillets/box. Thermocouples inserted in the fillet of a centrally placed tray pack in each box measured tempera-

ture. Each cyclic TA consisted of the pallet kept at 4°C for 2 hours followed by moving it to 23±2°C for 2 hours for a total of 6 cycles (24 hours). A box kept at 4°C acted as control. Baseline APC and psychrotrophic plate count (PSY) sampling was conducted before and after the 24-hour study (1 fillet x two trays x 9 boxes). After the 24-hour TA, tray packs from selected boxes were placed on shelves at 4°C to simulate retail conditions for 8 days and sampled (3 trays/box) for APC and PSY every 2 days. Data was analyzed using one-way ANOVA to determine significant differences between the means at p<0.05 (Tukey’s HSD). Monte Carlo predictive models were developed using temperature profile and microbiological counts of each box. Cyclic TA boxes reached spoilage (7 logs CFU/mL) at 2.5 days before the control box (p<0.05) indicating the effects of TA on increased spoilage. Models predicted that the boxes in the top and bottom layer had a higher risk of crossing 4°C as compared to the middle layers while specifying the box with the highest risk. The “first-expire first-out” models predicted the remaining shelf-life of the raw chicken stored in each box. The experiment emphasizes the need for increased temperature monitoring throughout the last mile of cold-chain and that the “First-in, First-out” model may need to transition to a “First-expire, First-out” model.

Key Words: Temperature Abuse, Supply Chain, APC, Psychrotroph, Shelf-life

P224 Comparison of leg parameters in embryonic chicks from three broiler lines and an inbred Junglefowl line. Annie Lozano^{*1GS}, Monica Franco¹, Sara Orłowski², Katy Tarrant¹ ¹*California State University, Fresno*, ²*University of Arkansas*

Intensive genetic selection in broiler chickens has resulted in a measurable impact on production traits such as live weight and feed efficiency. While selection has promoted continual progress, maintaining an understanding on the changes of factors, such as the skeletal integrity, are critical. This study aims to better understand how selection has played a role in the leg development of broilers and a direct ancestor during one time point in embryological development. In this study, leg parameters were measured in four genetic lines housed at the University of Arkansas: a random bred modern broiler line from 2015 (MRB), a random bred broiler line from the 1995 (95RAN), Athens Canadian Random Bred from the 1950s (ACRB) line, and an inbred Junglefowl line (JF). Twenty-five eggs from each line, totaling 100 fertilized eggs, were removed from the incubator at E 15 and humanely euthanized. Chicks were removed from the egg, weighed, and the left leg was disarticulated. Weight and length parameters were taken, averaged, and an ANOVA and Tukey’s HSD were conducted in JMP v.16 to compare leg parameters amongst lines. Bird weight of the MRB line were significantly larger than all other lines at 15.64 ± 0.29 g, followed by the 95RAN, ACRB, and JF lines (P < 0.0001). A similar trend was seen for the weight of the legs (P < 0.0001). Interestingly, the weight of the femur in the MRB line (0.013 ± 0.0005 g) was not significantly larger than that of the 95RAN line (0.012 ± 0.0005; P = 0.83). Ultimately, the ratio of the femur weight to total leg weight of the 95RAN line birds was larger than all other lines at 0.115 ± 0.0004. Combining the data from this study with previous studies, evaluating leg parameters in broiler lines throughout grow-out, provides insight as to how the leg parameters develop and change throughout different stages of development.

Key Words: broiler, junglefowl, random bred

P225 Effect of application method of a formaldehyde-based feed antimicrobial on efficacy and safety Dillon Mellick^{*}, Adam Cannon, Arlene Lamprey, D.J. Wolett, Brenda Reid, Stephen Klein *Kemin Industries*

Application and mixing are key components to the safe and efficacious use of formaldehyde-based feed antimicrobials. Initial distribution of the product is critical, and selecting a spray nozzle that consistently provides the greatest distribution lessens the requirements of the feed mixer to perform secondary mixing. Two types of nozzles were tested to determine the mixing efficiency, droplet size, formaldehyde exposure and ability of Sal

CURB® (SC) to prevent *Salmonella* contamination, under simulated feed mill conditions. Treatments consisted of two nozzle types, hydraulic (HD) and air assist (AA), with three varying air pressures (standard = 3-5, high = 7, and low < 1 PSI). Four replicate mixings for each treatment were conducted. Five samples from each mixing were analyzed for propionic acid to determine SC level and calculate mixing coefficient of variation. Droplet size was calculated by the nozzle supplier, and formaldehyde levels were measured using a handheld formaldehyde meter during liquid application, mixing, and sampling. Samples underwent a *Salmonella* challenge to determine the ability of SC to prevent *Salmonella* contamination at 10^4 CFU/g. The HD nozzle and the AA nozzle at high pressure had the best mixing efficiency ($2.84\% \pm 1.77$ and $2.75\% \pm 1.21$ respectively), followed by the AA nozzle at standard pressure ($4.23\% \pm 0.33$) and AA nozzle at low pressure ($7.37\% \pm 4.30$). The smallest droplet size was observed in the AA high pressure and followed by standard pressure (60.8 microns ± 4.5 and 118.0 microns ± 4.2 respectively). The HD nozzle had a droplet size of 220.8 microns ± 1.5 and the AA low pressure had the largest droplet size (243.5 microns ± 11.8). The lowest formaldehyde levels were recorded for the AA nozzle with low pressure and the HD nozzle (0.09 ppm ± 0.05 and 0.14 ppm ± 0.06 respectively). The AA nozzle at standard and high pressure had elevated formaldehyde levels of 0.3 ppm ± 0.21 and 0.7 ppm ± 0.87 . SC was able to prevent a *Salmonella* contamination regardless of nozzle type. In this study, the HD nozzle provided the most efficient mixing, while also maintaining low formaldehyde levels in the ambient environment. Both nozzles can be effective when used under proper air pressures to distribute formaldehyde-based products.

Key Words: Salmonella, Feed, Formaldehyde, Spray Nozzle

P226 The effects of potassium ferrate (VI) and water rinse on the pH and color of chicken breast meat Wendy Attuquayefio*, Michael Barnas, Skyler Lewis, James McNaughton *AHPharma Inc.*

Potassium Ferrate (VI) (CAS#: 13718-66-6) is a potent oxidizing agent used primarily in water and wastewater treatment. The use of ferrate (VI) for disinfecting poultry carcasses and wastewater during processing is being explored. However, there is concern that potassium ferrate (VI) may impact chicken meat quality, and studies on the effect of potassium ferrate (VI) on chicken meat quality are limited. The objective of this study was to determine the impact of ferrate (VI) dip and water rinse on chicken breast meat quality. Approximately 300 g of boneless, skinless chicken breast meat (N=3 per treatment) were dipped in deionized water (Control) and varying concentrations of potassium ferrate (VI) (0.038%, 0.075%, 0.15%, 0.3% and 0.6%) for 5 min and rinsed with water for 5 min. The quality parameters assessed on the breast meat were color (lightness (L*), redness (a*) and yellowness (b*)) and pH. Data were analyzed using the generalized linear model (GLM) procedure of SAS, and the treatment LSmeans were compared using 2-way anova with PDIF option respectively ($P < 0.05$). Ferrate (VI) had a significant effect on color and pH. The 0.15%, 0.3% and 0.6% ferrate concentrations significantly decreased L* value and increased a* and b* values of chicken breast meat. The L* values of the breast meat dipped in 0.3% and 0.6% ferrate solutions were still significantly different from that of the control after rinsing in water. Rinsing did not significantly change the a* value of breast meat, except for those dipped in 0.3% ferrate. Rinsing also significantly decreased the b* value of breast meat dipped in 0.075%, 0.3% and 0.6% ferrate solutions. Chicken breasts dipped in 0.15%, 0.3% and 0.6% ferrate solution had a significantly higher pH compared to the control. Rinsing did not significantly affect the pH of chicken breasts dipped in 0.15% and 0.6% ferrate solution. Overall, ferrate (VI) appeared to affect both color and pH of chicken meat at higher concentrations. Ferrate concentrations lower than 0.3% might be ideal in disinfecting breast meat during processing without significant changes in pH and color of meat. Furthermore, rinsing poultry carcasses in water after disinfection with ferrate (VI) could minimize changes in pH and color of chicken meat during processing.

Key Words: Quality, Ferrate, Chicken, Disinfection, Wastewater

Pathology

P230 Duck viral enteritis in domestic Muscovy ducks from an Ohio backyard flock Emma Kline^{*1GS}, Gavin Hitchener² ¹*Cornell University College of Veterinary Medicine*, ²*Cornell University Duck Research Laboratory*

This report describes the diagnosis of duck viral enteritis (DVE) or “Duck Plague” in two Muscovy ducks selected from a mixed genus (*Anas* and *Cairina*) flock experiencing sudden die-off in only its Muscovy (*Cairina moschata*) ducks. In July of 2021, two juvenile, domestic Muscovy ducks (*Cairina moschata*) were submitted to the Cornell University Duck Research Laboratory for necropsy. These domesticated ducks and wild waterfowl shared a common waterway and the owner reported finding several dead swans around the same time as her flock mortality began. Necropsy findings included necrotizing esophagitis in both ducks and hepatitis and severe necrotizing cloacitis in one duck (duck 2). Mild annular band necrosis was noted in both birds. Microscopically ducks had classic necrotizing erosive and ulcerative mucosal changes in the esophagus and multifocally throughout the gastrointestinal mucosa. Necrotizing hepatitis and rare intranuclear viral inclusion bodies were evident in multiple tissues. Molecular diagnostics performed at the NY Animal Health Diagnostic Center recovered DVE virus in pooled spleen and esophagus from Duck 2. Both ducks had substantial trematode/schistosome infections with obliterative endophlebitis-associated changes in the intestinal serosal sections, and duck 2 had trematode eggs present in the cerebrum. Gastrointestinal findings in these ducks included obliterative endophlebitis due to adult schistosomes as well as villous atrophy and enteritis due to the migration of schistosome eggs. These changes are a significant comorbidity that likely enhanced disease progression in these birds. Duck virus enteritis affects ducks, geese, and swans with potential for high morbidity and mortality and poses a threat to domestic duck farms as well as wild waterfowl. Commercially available vaccines and enclosed housing of ducks have

made both commercial and wildlife-associated incidences of Duck Plague rare in North America.

Key Words: duck viral enteritis, pathology, duck

P231 Tau cells of ducks – a plasmacyte form with a convoluted nucleus Paul Cotter^{*1}, Ben Fetrow² ¹*Cotter Laboratory*, ²*Maple Leaf Farms*

The purpose is to describe atypical plasmacytes (PC) of duck blood found between ages of 4 and 6 wk (N=93). The method is by a light microscopy of blood films stained with Wright-Giemsa. Samples were obtained at commercial farms and prepared within 24 hr of collection. The data are obtained from SDC of 200 cells/slide at 40x magnification. Representative cells were photographed at 100x (oil) magnification. Some examples are presented in the context of cells of another series. The results, 138 cells with convoluted nuclei were grouped by lobe as: 2 lobe N = 54, 3 lobe N = 52, 4 lobe N = 14, 5 lobe N = 12, 6 lobe N = 5, 7 lobe N = 2, and 8 lobe N = 1. The correlation between lobe number and cell area is ($y = 0.3x + 68$; $R^2 = 0.2$). Cells with 3 nuclear lobes, the first of the study to be detected, were in a form resembling the letter “tau” so it is used as a descriptor. Examples having 2 – 8 nuclear lobes are shown in comparison with “standard” plasma cells and Mott-types. The reason for variable lobe number is unknown, however tau-types occurring in multiple myeloma (MM) are associated with aggressiveness. It may be that a convoluted nucleus allows more flexibility, facilitating cell passage through the endothelial linings of blood vessels contributing to metastasis. As tau cells are not malignant, as are MM cells, this explanation may not apply. Alternately nuclear flexibility may enhance the capacity of a tau-plasmacyte to access a diseased tissue. Conclusion/discussion; a circulating PC suggests a complex hemogram. Recognizing a cell as a PC relies heavily on the presence of an eccentric nucleus containing a “cart-wheel” chromatin configuration. A Mott-type PC has a “grape-cell” cytoplasmic configuration as a con-

sequence of distention of the endoplasmic reticulum due to accumulated antibody. It is not known if the atypical nuclear convolution of tau cells is also associated with some sort of secretory defect. A conspicuous Hof (Golgi) is seen in some tau cells but others lack this feature. Some PC of bone marrow are able to divide by mitosis and amitosis. If a tau PC retains a mitotic capacity or is restricted to divide by amitosis is unknown. The recognition of a tau cell as a member of the PC series may contribute to a better interpretation of the SDC. The purpose is to describe atypical plasmacytes (PC) of duck blood found between ages of 4 and 6 wk (N=93). The method is by a light microscopy of blood films stained with Wright-Giemsa. Samples were obtained at commercial farms and prepared within 24 hr of collection. The data are obtained from SDC of 200 cells/slide at 40x magnification. Representative cells were photographed at 100x (oil) magnification. Some examples are presented in the context of cells of another series. The results, 138 cells with convoluted nuclei were grouped by lobe as: 2 lobe N = 54, 3 lobe N = 52, 4 lobe N = 14, 5 lobe N = 12, 6 lobe N = 5, 7 lobe N = 2, and 8 lobe N = 1. The correlation between lobe number and cell area is ($y = 0.3x + 68$; $R^2 = 0.2$). Cells with 3 nuclear lobes, the first of the study to be detected, were in a form resembling the letter "tau" so it is used as a descriptor. Examples having 2 – 8 nuclear lobes are shown in comparison with "standard" plasma cells and Mott-types. The reason for variable lobe number is unknown, however tau-types occurring in multiple myeloma (MM) are associated with aggressiveness. It may be that a convoluted nucleus allows more flexibility, facilitating cell passage through the endothelial linings of blood vessels contributing to metastasis. As tau cells are not malignant, as are MM cells, this explanation may not apply. Alternately nuclear flexibility may enhance the capacity of a tau-plasmacyte to access a diseased tissue. Conclusion/discussion; a circulating PC suggests a complex hemogram. Recognizing a cell as a PC relies heavily on the presence of an eccentric nucleus containing a "cart-wheel" chromatin configuration. A Mott-type PC has a "grape-cell" cytoplasmic configuration as a consequence of distention of the endoplasmic reticulum due to accumulated antibody. It is not known if the atypical nuclear convolution of tau cells is also associated with some sort of secretory defect. A conspicuous Hof (Golgi) is seen in some tau cells but others lack this feature. Some PC of bone marrow are able to divide by mitosis and amitosis. If a tau PC retains a mitotic capacity or is restricted to divide by amitosis is unknown. The recognition of a tau cell as a member of the PC series may contribute to a better interpretation of the SDC.

Key Words: atypical plasma cell, duckling, tau-cell, SDC

P232 Effect of atmospheric ammonia and hardwood dust on the performance and respiratory integrity of broiler chickens Dorian Dominguez*, Daniel Hofstetter, Vinicius Buiatte, Alberto Gino Lorenzoni
The Pennsylvania State University

Wood byproducts such as sawdust are broadly used as bedding for poultry. Pennsylvania is the biggest producer of Red Oak (*Quercus rubra*) in the country. This specific type of hard wood contains high levels of toxic resins that are proven to be harmful to humans and animals after long exposure. Chronic inhalation of dust combined with common poultry pollutants such as ammonia gas may cause damage to airways and reduce the performance of chickens. Our study aimed to assess the inflammatory and productive response of broilers exposed to pure hardwood dust and ammonia. 96 day-old broilers were raised under the same environmental and nutritional conditions. Birds were randomly divided into 4 environmental chambers to receive one of four treatments: 1, negative control with no intervention; 2, birds exposed to pure red oak wood dust; 3, birds exposed to 50 ppm ammonia; 4, birds exposed to a combination of ammonia and pure red oak wood dust. At the end of the experiment (42 days of age), we evaluated body weights, feed conversion, liver and spleen relative weight. Histologic samples of nasal turbinates, trachea, and lung were blindly analyzed to measure the tracheal mucosal thickness and to determine histopathological lesion scores (0 to 4). No differences were found on body-

weights, feed conversion, tracheal mucosal thickness, and trachea lesion score ($p > 0.05$). Liver relative weight was higher ($p < 0.05$) in birds of the negative control, compared to birds in the other treatments. The nasal turbinates and lung lesion scores were lower ($p < 0.05$) in birds of the negative control, compared to other birds in the other treatments. Despite the steady exposure to the dust and ammonia birds showed only a mild inflammatory effect on the respiratory tract and no effects on productive parameters.

Key Words: Chicken, Wood, Dust, Ammonia, Performance

P233 Autophagy Machinery is Dysregulated in Bacterial Chondronecrosis with Osteomyelitis (BCO) broilers Alison Ramser^{*1,2}, Elizabeth Greene¹, Robert Wideman¹, Adnan Alrubaye^{1,2}, Sami Dridi^{1,2}
¹University of Arkansas, Center of Excellence for Poultry Science, ²University of Arkansas, Cell and Molecular Biology

Autophagy is characterized by lysosomal degradation of cellular components and foreign bodies in order to maintain cellular homeostasis and improve cell survival. Indeed, autophagy has a role in bone homeostasis, skeletal diseases, and bacterial infections as both a cell-survival and cell-death pathway. This study sought to determine the role autophagy could play in bacterial chondronecrosis with osteomyelitis (BCO). BCO is a bacterial infection of mechanically stressed leg bone growth plates which often leads to lameness. Gene expression analysis showed a significant downregulation of genes coding for autophagy machinery involved in every stage of autophagy in BCO-affected bone. This included ATG13, SQSTM1 (p62), ATG9B, ATG16L, ATG12, LC3C, and RAB7A. Additionally, protein expression for LC3 was also significantly lower in BCO compared to healthy birds. Human fetal osteoblast cells (hFOB) challenged with a known BCO isolate showed a similar dysregulation of autophagy machinery coupled with a significant decrease in cell viability. Blocking autophagy with 3-MA or chloroquine (CQ), rescues osteoblast cell viability during bacterial challenge. Together, these results are the first to implicate autophagy machinery manipulation by bacteria as part of BCO pathogenicity.

Key Words: lameness, broiler, BCO/FHN, autophagy, bone

P234 Evaluating the accuracy of virulent genes model in predicting the source of extraintestinal *Escherichia coli* isolates, collected from colibacillosis infected or healthy layer chicken Fozol Ovi^{*GS}, Li Zhang, Pratima Adhikari
Mississippi State University

Previous research suggested that avian pathogenic *Escherichia coli* (APEC) and avian fecal *E. coli* (AFEC) can be classified by a predictor model accounting the presence of 5 virulent genes: *iroN*, *ompT*, *hlyF*, *iss* and *iutA*. This study aims to classify extraintestinal *E. coli* collected from two sets of layer hens: healthy or diagnosed with field cases of colibacillosis, by evaluating the presence of those 5 genes. A total of 58 *E. coli* was isolated, in which 42 were isolated from lungs, liver, peritoneum and pericardium of healthy layers. The remaining 16 were isolated from colibacillosis infected hens previously diagnosed by a registered veterinarian. These isolates were confirmed as *E. coli* in a PCR test by identifying the presence of *ybbw* gene. A pentaplex PCR was performed on both types of samples to evaluate the presence of 5 virulent genes. Data were statistically analyzed using Fisher exact test. A Linear discriminant analysis (LDA) was performed to classify the source of those isolates. Results showed the *ompT* gene has a significantly higher frequency in isolates from colibacillosis hens than healthy hens (100 vs 72.41%, $P = 0.01$), which indicates this gene is highly associated with establishing colibacillosis in layers. Significantly higher proportion of isolates from colibacillosis infected hens had all 5 virulent genes present than isolates from healthy hens (81.25 vs 47.62%, $P = 0.01$). LDA correctly classified 14 out of 16 isolates from colibacillosis infected hens based on 5 virulent genes with an accuracy of 87.50%. Whereas, out of 42 isolates from healthy hens, only 22 were correctly classified with an accuracy of 52.38%. Thus we conclude that virulence estimation of APEC isolates based on these 5 genes may not ac-

curately represent the actual pathogenicity in layer chicken. Other underlying factors like hen's immunity and stress condition may compound the ability of an APEC isolate to cause colibacillosis in 37.93% of the cases. Therefore, a correlation between the predicted virulence of an isolate and its in-vivo pathogenicity in layer hen needs to be established. Identifying other virulent genes with a differential prevalence between the extraintestinal *E.coli* isolates of healthy and colibacillosis infected hen may improve this correlation.

Key Words: APEC, Genotype, Pathogenicity, Pentaplex, Virulence

P235 Diagnosis of osteopenic and osteomegalic diseases in broilers submitted to different vitamin D3 dietary levels Tainá Lopes^{*GS}, Mariana Vasconcelos, Isadora Araújo, Lorena Sousa, Bruno Bertassoli, Natália Ocarino, Leonardo Lara, Rogeria Serakides, Itallo Araújo *Universidade Federal de Minas Gerais*

The cholecalciferol (VitD3) supplementation in broiler's diets has been used to prevent bone problems but, the supplementation levels vary according to the reference. The Nutritional Research Council (NRC) recommends 200 IU of VitD3/kg for all phases (NRC, 1994) but, higher levels are indicated by the Cobb strain guide, which recommends 5.000 IU/kg of feed for all phases (Cobb, 2018). This report describes the diagnosis of osteopenic and osteomegalic diseases arising from different VitD3 dietary levels. A total of 360 male 1-old-day Cob500® broiler chickens were distributed between 5 treatments with 6 replicates of 12 broilers each. Dietary treatments were: 1) 100%: 2.500 IU/kg starter/2.000 IU/kg grower. 2) 75%: 1.875 IU/kg starter/1.500 IU/kg grower. 3) 1.250 IU/kg starter/1.000 IU/kg grower. 4) 625 IU/kg starter/500 IU/kg grower. 5) 0 IU/kg in both phases. At 35 days old, one broiler from each replicate was euthanized to collect the left tibia. The samples were fixed in 10% buffered formalin, descaled, processed by routine paraffin embedding technique, and subjected to HE staining. The tibias of broilers from the 0% group presented histological alterations compatible with rickets, generalized fibrous osteodystrophy, and osteochondrosis. In the 25% and 50% groups were found histological alterations compatible with rickets and generalized fibrous osteodystrophy. Only broilers from the 75% group showed normal tibial development without histopathological changes. The broilers supplemented with 100% of VitD3 level presented unexpected observations such as an asynchrony in the process of chondrocyte differentiation (tibial dyschondroplasia) and an excessive increase in the amount of bone tissue (osteopetrosis). In this group, tibial dyschondroplasia and osteopetrosis were likely a manifestation of hypercalcitoninism. The histopathological findings demonstrate that the use of 1.875 IU of VitD3/kg in the starter phase and 1.500 IU of VitD3/kg in the grower phase promote bone health. The diagnosis of bone diseases such as osteopetrosis and tibial dyschondroplasia in broilers fed with diets without alteration in the level of minerals and using VitD3 levels equal or lower than those recommended have been rarely described and needs to be better investigated.

Key Words: tibial dyschondroplasia, osteopetrosis, fibrous osteodystrophy, bone health

P239 Onset of Immunity of a Recombinant HVT-IBD-ND Vaccine Against a Classic IBDV, a Very Virulent IBDV, and Two Velogenic NDV challenges Sing Rong*, Tura Bru, Kelly Turner-Alston, Candyce Pacione, Lauren Taylor, Rut Vila, John Dickson, Jennifer Embrey, Alicia Molas *Zoetis - VMRD*

A recombinant HVT-IBD-ND dual vector vaccine was developed for protection against infectious bursal disease (IBD), an acute and highly contagious viral infection of young chickens that causes immunosuppression and increased susceptibility to other infectious agents; Newcastle disease (ND), a highly contagious and fatal disease affecting all species of birds; and Marek's disease (MD), a common cause of condemnations and immune suppression in broilers and tumors/mortality in older birds. The objective of the following four studies was to evaluate the early onset of immunity to ND and IBD provided by the vaccination with the novel

HVT-IBD-ND recombinant vaccine. In the first study, birds were vaccinated by *in ovo* on E18 at the minimum protective dose, and then challenged with a classic IBDV on either Day 14 or Day 21 of age. Protection of 90% (18/20) was observed for both Day 14 and Day 21 challenges, while control groups had 0% (0/20) or 5% (1/20) protection, respectively. In the second study, SPF leghorn chickens that were vaccinated subcutaneously at hatch were challenged on Day 14 with a very virulent IBDV. Protection of 98% (39/40) was observed for the vaccinated group compared to 0% (0/40) for the control group. The mortality was 2.5% (1/40) for the vaccinated group compared to 78% (31/40) for the control group. In the third study, SPF leghorn chickens were either vaccinated with recombinant HVT-IBD-ND *in ovo* on E18, or subcutaneously on day of hatch. On Day 21 of age, birds were challenged with a velogenic NDV Herts Weybridge 33/56. Protection of 96% (25/26) was observed for both *in ovo* and subcutaneously vaccinated groups compared to 0% (0/15) protection for control group. In the fourth study, birds were challenged with a velogenic NDV Texas GB on either Day 14 or Day 21 of age following *in ovo* vaccination on E18. Protection of 75% (15/20) was observed for Day 14 challenge and protection of 95% (19/20) was observed for Day 21, compared to 0% (0/20) protection for control groups for each challenge.

Key Words: Vector Vaccine, IBDV, NDV, Marek's disease, Onset of immunity

P240 Evaluation of protection by ND vaccination protocols against early challenge with Velogenic Newcastle virus-VII.1 Wael Elfeil¹, Mahamed Rady², Walid Kilany², Ahmed Sedeik², Magdy Elkady³, Magdy Elsayed^{4,5} ¹Suez Canal University, ²Agriculture Research Center, ³Beni-Suef University, ⁴Cairo University, ⁵Middle East for Veterinary Vaccine Company (MEVAC)

The aim of this work was to evaluate effectiveness of three different vaccination regime (G-1 double inactivated ND vaccine at 1/10 day of age, G-2 single inactivated ND vaccine at 7-day of age and G-3 single inactivated ND at one-day of age) in-combination with three-dose from live ND vaccine against challenge with velogenic NDV (vNDV). 180,000 one-day old chicks obtained from commercial hatchery placed in three commercial broiler station "60,000 birds/station" and 40 one-day old chicks moved to Biosafety level-3 isolators (BSL-3) at MEVAC facility to serve as control groups (non-vaccinated group challenged "G-4" and non-vaccinated non-challenge-G-5). On weekly basis blood samples, cloacal swabs and oropharyngeal swabs collected check develop of Humeral immune response and exposure to any life-threatening Respiratory virus (Avian influenza" AIV", NDV and infectious Bronchitis "IB" virus). At 24-day of age 25 birds from each station (G1-3) moved to BSL-3 and kept under observation for 36 hours; cloacal and oropharyngeal swabs collected and three birds from each group euthanized, and internal organs examined for three repeated times with 12 hours interval to ensure that birds free from any live threatening viral respiratory pathogen (AIV, NDV, IB). Birds in G1-4 challenged with vNDV VII.1 (10^6 EID₅₀) in 0.5 ml/ bird PSB via intranasal route and birds in G-5 received 0.5 ml PBS via intranasal route. Birds in G1-5 kept in BSL-3 for 10 days under observation and oropharyngeal swabs collected on 3,6,9 days post challenge (dpc). Regarding protection virus against mortalities following challenge with vNDV-VII.1; were 100% (12/12), 100% (12/12), 91.7% (11/12), 0% (0/12) and 100% (12/12) in groups 1-5 respectively. Regarding virus shedding birds in G-1 showed significant lower shedding virus (amount of virus shedding and number of shedding in comparison to G2/3). In conclusion, using NDV inactivated vaccine in two-dose regime with live vaccines can provide protection to commercial broiler chicken against early challenge with vNDV in endemic areas as early as 26 days of age with significant lower shedding rate and the application of such regime can be effective tool in control NDV under vaccination strategy.

Key Words: Newcastle virus, early challenge, commercial broilers, maternal antibodies, field challenge

P243 Evaluation of circulatory mRNA abundance of pro-inflammatory and regulatory cytokines and receptors during a subclinical necrotic enteritis challenge Laney Froebel^{*GS}, Nima Emami, Rami Dalloul *University of Georgia*

Necrotic enteritis (NE) is an enteric disease typically caused by toxins produced by *Clostridium perfringens* (Cp) and is estimated to cost the global poultry industry 6 billion USD annually. In this study, we evaluated the effects of NE induced by two different Cp strains (Cp#4 and Cp#6) during an infection model with *Eimeria maxima* (EM) on the circulatory mRNA abundance of pro-inflammatory and regulatory cytokines and receptors of broilers. A total of 33 day (d)-old broiler chickens were individually wing tagged and allocated to one of four treatment groups: non-challenged control (NC; n=4), EM only challenge (EM; n=9), EM + Cp#4 (Cp4; n=10), and EM + Cp#6 (Cp6; n=10). On d 14, birds were orally gavaged with approximately 3,000 EM sporulated oocysts. Birds in Cp4 and Cp6 groups were orally gavaged with approximately 1×10^8 CFU of Cp on d 19 and d 20. On d 21, blood was collected from all birds to measure mRNA abundance of interleukin (IL)-10, IL-1 β , interferon (IFN)- γ , TNF receptor-associated factor (TRAF)-5 and TRAF6. Data were analyzed using ANOVA (JMP Pro 16) and significance ($P \leq 0.05$) between treatments was evaluated by LSD test. The Cp6 birds exhibited greater ($P < 0.05$) mRNA abundance of IL-10 and IL-1 β compared to Cp4 and EM birds. Analysis of mRNA abundance of IFN- γ , TRAF5, and TRAF6 showed no statistical differences between the four treatment groups. While TRAF5 and TRAF6 mRNA abundance was not significantly different, their regulation at the protein level might induce pro-inflammatory and regulatory cytokines. Collectively, NE induced with Cp#6 resulted in a stronger pro-inflammatory response in the circulation compared to Cp#4. The localized responses should be evaluated at the inflicted sites to better evaluate their potential correlation with the mRNA levels observed in circulation.

Key Words: Necrotic enteritis, *Clostridium perfringens*, *Eimeria maxima*, subclinical, cytokine

P244 Histomoniasis associated bursal pathology in turkeys Vijay Durairaj¹, Mary Drozd², Deborah Higuchi¹, Ryan Vander Veen¹
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Histomoniasis is an economically important disease of turkeys associated with high mortality and morbidity. Histomoniasis causes systemic infection in turkeys and causes distinctive lesions in liver and ceca. The main objective of this study was to evaluate the effect of two wild-type *H. meleagridis* isolates (HMA and HMB) on the bursa of Fabricius as any compromise to the bursa of Fabricius adversely affects the humoral immunity. Two-week old poults (n=19) were enrolled in this study. Five birds were unchallenged and fourteen birds were challenged by intra-cloacal administration with either HMA or HMB. All poults were commingled and raised in an isolator with necropsy performed nine days post-challenge. Histomoniasis associated gross lesions were observed in all challenged birds. Microscopic cecal lesions included one or more of the following: mucosal ulceration, histiocytic and heterophilic inflammation associated with *Histomonas* organisms, edema, and transmural necrosis. Microscopic evaluation of bursa of Fabricius included multifocal, *H. meleagridis* associated bursitis, as well as variable lymphoid depletion, and lymphoid hyperplasia. Both wild-type *H. meleagridis* isolates adversely affected bursa of Fabricius.

Key Words: Histomoniasis, Blackhead disease, Turkeys, Pathogenesis, Bursa of Fabricius

P245 RNA-Seq analysis of local host response to *Eimeria maxima* infection in broiler chickens Ahmed Ghareeb¹, James Foutz¹, Gustavo Schneiders², Marie Milfort¹, Alberta Fuller¹, Romdhane Rekaya³, Samuel Aggrey¹ ¹Department of Poultry Science, University of Georgia, ²Merck & Co., Inc., ³Department of Animal and Dairy Science, University of Georgia

Eimeria (E.) maxima (EM) causes coccidiosis in chickens. It infects the jejunum and ileum damaging the luminal mucosa and impacts digestibility and absorption, leading to high morbidity and mortality. Our objective was to elucidate ileum transcriptional changes in response to EM infection. One hundred and twenty 21-day-old males Ross 708 were randomly allocated into two groups each of 6 cages and 10 birds per cage: Uninfected control (C) group was received water, and the treatment (EM) group were gavage 200 x 10³ sporulated EM oocysts/bird. Chickens were fed on *ad libitum* unmedicated standard grower diet. At 6 day-post infection, five chickens per group were randomly sampled, and ileum tissues were collected for RNA extraction and then sequenced using NGS Illumina sequencing platforms. The high-quality reads were processed, and the hit counts were used for identifying the differentially expressed genes (DEGs) for each treatment using DESeq2 at FDR of 0.05. Gene ontology (GO) with GlueGo integrated terms, and KEGG pathway were used for DEGs functional analysis and pathways clustering and identification. A total of 3,377 DEGs (1,677 upregulated and 1,700 downregulated) were determined in the EM group compared with the C group at fold change \geq 1.2. Pathways related to pathogen pattern recognition receptors, cytokine interaction and innate and adaptive immune responses, focal adhesion pathway and junction proteins pathways were enriched in the EM group. The energy production and carbohydrate pathways, including the citrate cycle, pentose phosphate, oxidative phosphorylation, and Glycolysis/Gluconeogenesis were downregulated in the EM group. Pathways related to transporter systems and lipid signaling/metabolism, such as ABC transporters and PPAR signaling, respectively, were also downregulated in the EM group. The molecular immune functions were enhanced to encounter the parasitic invasion. However, the analysis was conducted at 6 dpi, the enrichment of the primary immune responses, such as PRR, was still prominent. Junction proteins pathways were upregulated to compensate for the permeability induced by *E. maxima* infection. The ileum cells lose their regular metabolic and absorption functions due to the parasitic pressure.

Key Words: *Eimeria maxima*, ileum, transcriptome

P246 The effect of a whole yeast product and two plant extracts supplemented alone or combined on broiler performance, lesions scores, and oocyst shedding during an experimental coccidia challenge. Jose Charal¹, Chet Wiernusz², Brian Glover¹, Milan Hruby¹, Annalise Anderson¹ ¹ADM Animal Nutrition, ²NutriQuest

The study evaluated the effect of a whole yeast (*Pichia guilliermondii*, WYP) product (CitriStim®), a blend of capsicum and turmeric oleoresin (Xtract® Nature, XTN), and a single plant extract (SPX) fed alone or in combination, on broiler performance, lesion scores and oocyst shedding during an experimental coccidia challenge. Newly hatched (336 males) Ross 708 broilers were randomly assigned to eight treatments replicated in 7 floor pens with 6 birds per pen. The treatments included a Non-infected Control (Basal + mock-challenge; NIC), Infected Control (Basal + Coccidi challenge; IC), YIC (IC + 500 g/t of WYP), XIC (IC+100g/t of XTN), YXIC (IC + 500g/t of WYP + 100g/t XTN), SIC (IC + 68 g/t of SPX), YSIC (IC + 500/t of WYP + 68 g/t SPX), and XSIC (IC + 100 g/t XTN + 68 g/t SPX). On D14, birds in all the treatments, except the NIC (mock-challenged with distilled water), were challenged with Coccivac-B52 vac-

cine (1.3 x 10⁵ live oocyst/bird). The coccidiosis challenge model was effective at inducing gross lesion scores and oocyst shedding in the excreta and reducing body weight gain and feed intake. Compared to NIC, IC broilers had 18% and 10% reduction on ADG at 7 (P < 0.001) and 14 d (P < 0.05) post-challenge. Birds on YIC, XIC, YSIC, and XSIC treatments produced intermediate ADG during the same periods (P < 0.05). Similarly, a reduction of 10% BW was observed in IC birds (vs NIC) at 7 (P < 0.004) and 14 d (P < 0.004) after the challenge, birds on YIC, XIC, YSIC, and XSIC treatments produced intermediate BW during the same periods. A trend for higher overall BW was observed in broilers fed YIC treatment (P = 0.10). A trend for higher FCR was observed on challenged birds at 7 and 14 d post challenge, and FCR was lower on IC, YXIC, YSIC, and XSIC on d 28 – 32 compared to NIC (P < 0.05) birds. Overall adj-FCR trend to be lower in birds on YIC, XIC, and XSIC treatments (P = 0.20). Oocyst shedding was decreased on XSC birds (P < 0.05) 7 d post-challenge. Lesions in the upper and middle regions of the intestine were higher in challenged birds and no effect of feed additives at 7 d post-challenge (P < 0.05) was observed. These results suggest that WYP and plant extracts fed to broilers alone or in combination could ameliorate the impact of coccidiosis.

Key Words: Coccidia, Pichia yeast, Plant extracts, ADG, adjusted-FCR

SCAD

P248 Evaluation of the performance of different Nicarbazine-potentiated ionophores in the presence of a strict coccidia challenge

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This study had the purpose to compare the efficacy of nicarbazine-potentiated ionophores with a contemporary challenge of coccidia. These types of products have become ubiquitous in many markets around the world and certainly have become a cornerstone of the toolbox for coccidia control. The experimental model used called for a challenge of *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella* (214,000, 63,000, 7,700 oocyst/bird, respectively). The challenge was gavaged on Cobb500 male broilers at 18 days. Broilers were kept in floor pens separated in randomized complete block design for 5 treatments of 10 replicates per treatment. Two different nicarbazine-potentiated ionophores were used in this study. Two different doses were used for the NIC+SEM and NIC+NAR. Thirty birds per pen were distributed for a total of 1,500 birds. The nicarbazine-potentiated ionophore combinations were used up to 28 days. Semduramicin at 22.5 ppm was used after 28 days in all treatments. Lesion scoring for all treatments was performed at day 24 (3 birds per pen). The control group had no anticoccidials present in all feeds. Weight gain at the end of the trial was improved in all the treatment groups with anticoccidial. The higher doses of the anticoccidials had numerically lower weights. The treatments with NIC+SEM had the better weights at both doses and had significantly improved weights compared to any of the treatments with NIC+NAR. In regard to Feed Conversion, numerically, NIC+SEM improved FCR values, but statistical differences were not observed. The higher dose of NIC+NAR significantly impaired FCR compared to other treatments. On lesion scoring, there was a significant difference between the control group and all treatment groups regarding *E. acervulina*. This was not the same for *E. maxima* where lesion scoring was significantly higher. For both products, NIC+SEM and NIC+NAR, the higher dose (64+24 ppm and 70+70 ppm respectively) did not provide statistically significant improvement over their typical dose (48+18 ppm and 50+50 ppm respectively for the two products) neither in zootechnical performance nor in lesion scores, indicating no further improvement of the coccidiosis control by increasing the prevention doses over the typically recognized ones. Nicarbazine-potentiated ionophores remain the central pivot of coccidia control and the increased dose of these products did not bring any value in performance or lesion scoring.

Key Words: Anticoccidials, Semduramicin, Nicarbazine, Broilers

P249 Further analysis of computerized methods for *Eimeria* spp. enumeration

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Rapid non-invasive diagnostic tools for coccidia (*Eimeria* spp.) quantification and speciation that can be performed on-site are both challenging and necessary. We have worked with the software FIJI ImageJ (ImageJ) and more recently with Luna II machine, an automated cell counter, to aid in enumeration and speciation of coccidia oocysts.

Recently, we demonstrated that ImageJ was able to successfully count oo-cysts using images collected from prepared McMaster slides, especially when counts were greater than 400 oocysts per slide (or 20,000 OPG) and samples are free of debris. To improve the accuracy of the oocysts counts, the area and circularity of *E. mitis*, *E. acervulina*, *E. tenella* and *E. maxima* were specified separately in ImageJ. Results showed that ImageJ was even more accurate in counting oocysts in this way, while it was able to simultaneously identify the *Eimeria* species.

Although this method was proven to be accurate, its application at the barn-side is challenging, due to the need of having a camera installed over the microscope. Hence, we started working on using an automated cell counter that is traditionally used for cellular work. Results on the evalu-

ation of coccidia oocysts from a commercial chicken vaccine, using the Luna II cell counter, will be presented.

Key Words: *Eimeria*, enumeration, coccidia, computerized, OPG

P250 Characterization of vaccine-induced immune responses against coccidiosis in broiler chickens

Carissa Gaghan¹, Daniel Adams², Rocio Crespo¹, Kimberly Livingston³, Raveendra Kulkarni¹ ¹North Carolina State University, ²University of Georgia, ³Elanco

Coccidiosis in poultry, caused by *Eimeria* species, is a major enteric disease posing a significant challenge to the producer industry, particularly the broiler sector. Although commercial live vaccines are widely used for disease control, the vaccine-induced immune responses and the protective immune mechanisms are poorly characterized. The present study used a commercial broiler vaccine containing a mixture of *E. acervulina*, *E. maxima*, and *E. tenella* to vaccinate day-old chicks by spray followed by a challenge at 21 days of age with 20,000 oocysts of a mixture of wild type *Eimeria* species via oral gavage. Oocyst shedding, immune gene expression and cellular responses in the spleen and cecal tonsils were measured at pre- (days 14 and 21) and post-challenge (days 24, 28 and 35) time points. Results showed that the oocyst counts were significantly reduced in the vaccinated chickens at post-challenge compared to unvaccinated control group. Immune gene expression analysis showed that while there was a significant increase in the transcription of IFN γ , IL-12 and CD40 genes in the spleen and cecal tonsil tissues of the vaccinated birds at post-challenge, TLR21 gene expression in these birds was found significantly elevated at pre-challenge time point compared to controls. Cellular immunophenotyping analysis found that vaccination led to increased frequency of macrophages and activated CD4 and CD8 T cells in the spleen and cecal tonsils at pre and post-challenge time points. Furthermore, in-vitro stimulation of chicken macrophages (MQ-NCSU cells) with *E. acervulina*, *E. maxima*, and *E. tenella* showed a significantly increased expression of TLR21, TLR2 and IFN γ genes as well as macrophage production of nitric oxide when compared to unstimulated control groups. Based on these observations, three suggestions can be drawn; 1. Host recognition of *Eimeria* seem to operate via TLR21 and TLR2 receptors; 2. Coccidia vaccine induces a T helper-1 predominated protective response characterized by IFN γ and IL-12 cytokines and increased frequency of activated T cells in both local (cecal tonsil) and systemic (spleen) lymphoid tissues; 3. *Eimeria* can be recognized by macrophages via TLR2 and TLR21 receptors and that this recognition leads to their enhanced cellular activation status.

Key Words: coccidiosis, *Eimeria*, vaccine, immunity, immune response

P251 Survey of anticoccidial sensitivity and species of *Eimeria* affecting turkey farms in USA

Hafiz Abdullah^{*GS}, Audrey Duff, Kaylin Chasser, Johel Bielke, Kate McGovern, Michael Trombetta, Whitney Briggs, Lisa Bielke ^{The Ohio State University}

Presently, in the United States, a single commercial *Eimeria* vaccine is available for turkeys, which covers two species, *E. meleagris* and *E. adenoides*. Thus, flocks are at risk of wild-type infection even with vaccination programs in place. Resistance to anticoccidials can develop rapidly and options are limited for raised without antibiotics programs. Since 2019, samples from 150 commercial turkey flocks have been surveyed for *Eimeria* species and tested for anticoccidial sensitivity (TACS) to amprolium (0.024%), lasalocid (90.7g/ton), monensin (78g/ton), clopidol (125g/ton), halofuginone (2.72g/ton), and zoalene (170.3g/ton). Poults were raised on unmedicated feed in a single pen until 8d, when they were separated into 4 pens of 4 birds of each treatment (n=4/anticoccidial). On d10, poults were inoculated with 100 oocysts/bird, with a set of pens for non-treated control to compare oocyst output. Excreta was collected during peak shedding period for all species, d15 – 17, and counted in McMaster chambers to calculate relative oocyst output for all anticoccidials.

Samples from control pens were reserved for PCR identification using species-specific primers of the cytochrome c oxidase I gene. Samples with >80% reduction of output were considered sensitive, 30 – 79% reduction intermediate, and <30% reduction resistant based on probability for reproduction of disease. Of 43 samples that met TACS requirements, 14% were pan-sensitive while 26% were pan-resistant, showing sensitivity to ≤ 1 medication. Multi-drug resistant was classified as resistance to three or more anticoccidial compounds, and 62% of samples fell into this classification. Speciation indicates that *E. adenoides* and *E. meleagridis* had the highest prevalence, at 19/19 and 18/19 farms, respectively. *E. gallopavonis* was detected on 8/19 and *E. meleagridis* in 2/19 farms. While vaccination may be helpful for control of major species, others continue to persist. Options for control include commercial vaccines, autogenous vaccination, bioshuttle programs, and rotational or shuttle programs that include monitoring for anti-coccidial resistance. TACS indicate that high levels of resistance may be present in commercial flocks, highlighting the importance of monitoring resistance for poultry integrators.

Key Words: Eimeria

P252 Impact of dietary supplementation of Magni-Phi® Ultra on intestinal permeability during a coccidia challenge and feed restriction in broiler chickens Saheed Osho*, Kari Saddoris-Clemons, Brooke Humphrey, Miriam Garcia-Orellana *Phibro Animal Health Corporation*

Magni-Phi® Ultra (MPU; Phibro Animal Health, Teaneck, NJ) contains natural saponins from *Yucca schidigera* and *Quillaja saponaria*. Saponins have wide ranging biological effects of importance to the commercial poultry industry, especially antibiotic-free poultry production systems. Two experiments (Exp) were conducted to determine the effects of dietary MPU on intestinal permeability during a coccidia challenge (CC) and a feed restriction model. Intestinal permeability was assessed by measuring serum fluorescein isothiocyanate conjugated dextran (FITC-D). In Exp 1 and 2, a total of forty-two 9-d-old Ross 708 male broiler chicks were randomly allotted to 1 of 3 dietary treatments (7 replicate cages; 2 birds/cage) on d 9 post-hatch in a randomized complete block design. Diets were corn-soybean meal based and supplemented with no additive (CON), Salinomycin (66 ppm; SAL), or MPU (0.11 g/kg of diet). In Exp 1, all birds were orally gavaged on d 16 post hatch with 3× the recommended coccidia vaccine (Coccivac B52®) using 25 doses/kg BW. On d 5 post-challenge, all birds were orally gavaged with 8.4 mg FITC-D per kg BW and blood was collected 2 h later for serum FITC-D measurement. Fecal samples were collected on d 5 post-challenge for enumeration of oocysts per gram of feces (OPG). In Exp 2, experimental design and treatments were identical to Exp 1 except all birds had feed removed on d 21 for 10 h to induce intestinal dysbiosis. After 10 h of feed withdrawal, all birds were orally gavaged with 8.4 mg FITC-D per kg BW and blood was collected 2 h later for serum FITC-D measurement. Data in both Exp were analyzed with GLIMMIX procedure of SAS. In Exp 1, inclusion of MPU and SAL significantly improved ($P < 0.05$) BW gain and FCR compared to CON. Birds fed MPU had reduced OPG (67×10^3 vs. 130×10^3 OPG; $P < 0.05$) and serum FITC-D (61 vs. 109 ng/mL; $P < 0.05$) compared to CON. There were no differences in OPG or FITC-D levels between MPU and SAL ($P > 0.05$). In Exp 2, birds fed MPU or SAL had decreased ($P < 0.01$) serum FITC-D when compared to CON. In conclusion, MPU improved performance and alleviated the negative effects of leaky gut induced by both a coccidia challenge and dysbiosis in broiler chickens.

Key Words: Broiler chickens, coccidia challenge, feed restriction, Magni-Phi Ultra

P253 Prevalence of multi-serotype Salmonella populations in southeastern breeder flocks Amy Siceloff¹GS, Nikki Shariat¹, Doug Waltman² ¹*Department of Population Health, University of Georgia,* ²*Georgia Poultry Laboratory Network*

There is an increasing need to control *Salmonella* during poultry production as mitigation at processing is not sufficient. Successful control relies on a robust surveillance platform. Typical *Salmonella* surveillance identifies the most abundant serotypes in a sample and lacks the resolution to characterize multi-serotype populations. CRISPR-SeroSeq is an amplicon-based sequencing approach that uses the native CRISPR spacer sequences in *Salmonella* to quantify multiple serotypes in a sample.

Approximately 1,900 routine surveillance samples are collected from breeder flocks in the southeast and screened for *Salmonella* each month. Each week, an unbiased subset of *Salmonella* culture positive samples is analyzed by CRISPR-SeroSeq on the overnight tetrathionate enrichment broths. Briefly, total genomic DNA was isolated from the culture and CRISPR-SeroSeq performed in two sequential PCR steps: the first PCR targeted the CRISPR regions, and the second added dual index barcodes to facilitate multiplexed sequencing. DNA libraries were pooled in equimolar amounts and sequenced on an Illumina NextSeq.

Over nine months, we analyzed 202 samples. On average, traditional culture methods found a single serotype (1.1) per sample and found 24 different serotypes across the dataset. CRISPR-SeroSeq was able to detect an average of two serotypes (1.7) per sample and found 30 serotypes in total. In 39% (78/202), we detected two or more serotypes, and as many as seven serotypes were identified in a single sample. Kentucky was the most frequent serotype identified, present in 71% (144/202) samples. Other serotypes identified included Typhimurium and Infantis, which were detected in 12% (24/202) and 11% (23/202) of samples, respectively. Importantly, Infantis was not the most abundant in 87% (20/23) of these, suggesting it is outnumbered by other serotypes that are also present.

These results demonstrate that multi-serotype populations frequently occur in poultry. The increased resolution of CRISPR-SeroSeq reveals that traditional culture-based approaches underestimate *Salmonella* diversity within a sample. The data gleaned in this ongoing work will support a surveillance platform to monitor shifts in *Salmonella* serotype frequency and devise preemptive management strategies.

Key Words: Salmonella, CRISPR-SeroSeq, Breeders, Surveillance, Multi-serotype

P254 A laboratory evaluation of a tablet chlorine dioxide (Dutrition) to stabilized sodium chlorite in reducing Salmonella enteritidis in drinker lines Nathaniel Ollis¹, Charles Hofacre¹, Virginia Baxter¹, Dale Green², Chet Wiernusz² ¹*Southern Poultry Research Group,* ²*Nutriquest*

Drinking water sanitation is important for broiler health and for the reduction of salmonella and campylobacter colonization. Bacteria can produce a protective biofilm, therefore continuous sanitation is important to reduce bacterial growth. The poultry industry has used chlorine dioxide (ClO₂) by combining stabilized sodium chlorite and acid. To reduce complexity so two chemicals don't need to be mixed and to increase efficacy of drinking water antimicrobial products, a tablet chlorine dioxide product was developed. A comparison between stabilized sodium chlorite alone at 10 and 100 parts per million (PPM) and tablet chlorine dioxide (Dutrition) at 1 PPM was performed by exposing a nalidixic acid resistant *Salmonella enteritidis* to the products. The *Salmonella enteritidis* at 1×10^7 was ten-fold diluted in phosphate buffered saline to produce five ten-fold dilutions. The products were added to these tubes at a 1:1 ratio and vortexed. Each dilution was plated to xylose lysine tergitol agar with nalidixic acid every 5 minutes for 15 minutes, and incubated overnight at 37° C. The results from the salmonella isolation at each titration at each time (1, 5, 10, & 15 minutes) demonstrated sodium chlorite alone at 10 PPM ranged from 4.8×10^7 cfu/ml to 6.6×10^7 cfu/ml CFU/mL with an average of 45.896% re-

duction of salmonella. At an inclusion rate of 100 PPM stabilized sodium chlorite ranged from 3.19×10^7 cfu/ml to 5.60×10^7 cfu/ml with an average of 51.775% reduction of salmonella. The tablet chlorine dioxide at a rate of 1 PPM had salmonella growth only to 1.0×10^3 cfu/ml with a 99.999% reduction in salmonella. The non-treated control ranged from 5.86×10^7 cfu/ml to 1.08×10^8 cfu/ml. This laboratory model study demonstrated that the tablet chlorine dioxide (Dutrition) greatly reduced salmonella in the water while stabilized sodium chlorite alone had less effect on salmonella.

Key Words: Salmonella, Dutrition, Sanitation, Chlorine Dioxide, Sodium Chlorite

P255 Dietary inclusion of Diamond V Original XPC postbiotic is associated with reduced cecal Salmonella prevalence and loads in Honduran broilers William Chaney¹, Manuel Gutierrez², Abel Gernat¹, Arlene Fosmer¹, Timothy Johnson¹ ¹Diamond V, Cargill Health Technologies, ²Agrinvet Laboratorio

Feed additive technologies may provide additional benefit as interventions against foodborne pathogens of concern during primary poultry production. The purpose of this study was to evaluate postbiotic (Diamond V Original XPC) inclusion into the dietary ration of broilers and the subsequent potential reduction in cecal burden of *Salmonella enterica* over three cycles on a commercial farm in Honduras. Twelve houses within farm were matched by house features and assigned either the standard diet (CON) or standard diet plus the *Saccharomyces cerevisiae* fermentation-based postbiotic (SCFP). Fresh litter was placed in each study house prior to the first chick placements and retained in house for subsequent flock cycles. At a target market age of ~33-34 days, ceca from 25 birds were collected on-farm from each house, treatment, and cycle, and transported directly to a commercial laboratory for detection and enumeration of *Salmonella*. Outcomes were modeled and analyzed in SAS v9.4 with treatment differences determined significant at $p < 0.05$. Cecal prevalence of *Salmonella* was significantly lower in broilers fed SCFP (3.4%) as compared to CON (12.2%) ($P = 0.0006$). Within culture positive cecal samples, mean *Salmonella* loads were numerically reduced by 1.45 Log₁₀ MPN/g for SCFP (2.41 Log₁₀ MPN/g) over CON (3.86 Log₁₀ MPN/g) ($P = 0.121$). Estimated cecal *Salmonella* burden to processing was significantly lower for SCFP fed flocks (3.80 Log₁₀ MPN) compared to CON (7.31 Log₁₀ MPN) ($P = 0.003$). Under the conditions evaluated on this Honduran broiler farm, these data demonstrate that Diamond V Original XPC postbiotic is associated with prevalence, load, and overall estimated burden reduction of *Salmonella enterica* in broiler chickens and may be an effective pre-harvest intervention in a comprehensive food safety management plan.

Key Words: Broilers, Salmonella, Postbiotic, Preharvest Intervention, Food Safety

P256 Growth performance of broiler chickens after oral inoculation with different doses of Salmonella spp. Davis Fenster*, Candice Blue, Nima Emami, Laney Froebel, Jake Yarnall, Jinqian Wang, Harshavardhan Thippareddi, Rami Dalloul *University of Georgia*

Recent outbreaks of salmonellosis linked to poultry meat have raised concerns regarding the prevalence of *Salmonella* in such products and the potential risk of foodborne illnesses. Additionally, *Salmonella* bacteria can persist in poultry while birds are asymptomatic, which may negatively impact performance. The main objective of this study was to determine the effect of *Salmonella* spp. on performance of broiler chickens over a grow-out period (35 d). Day (d)-old male broilers (n=360) were weighed and separated into three groups (n=120/group) based on the *Salmonella* inoculation dose: (1) 1×10^2 , (2) 1×10^5 , or (3) 1×10^8 . Prior to placement, chicks were orally inoculated with a three-serovar cocktail consisting of equal numbers of *S. Typhimurium*, *S. Infantis*, and *S. Reading*. Each treatment group had six replicate pens (n=20/pen). Pen and feed weights were measured on d 0, 7, 14, and 35 to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). Mor-

talities were recorded and the performance parameters were adjusted accordingly. Performance parameters were analyzed by ANOVA using JMP (Pro 15) and significance ($P \leq 0.05$) between groups were detected by LSD test. From d 7 to 14, the ADFI for group 3 significantly increased compared to group 2 ($P = 0.038$). Over the course of the study, ADG and ADFI were not significantly different between the three groups. However, group 3 had a significantly higher ($P < 0.001$) cumulative FCR compared to groups 1 and 2. Based on these results, inoculating birds with 1×10^8 of a three-serovar *Salmonella* cocktail has a negative impact on FCR. Such a high dose might have negatively affected nutrient absorption in the small intestine or perhaps nutrients were directed towards generating immune responses to the bacterial load, which needs to be further evaluated.

Key Words: Salmonella, broiler, dose, serovar, performance

P257 Comparison of two live Salmonella Typhimurium vaccine programs in a commercial broiler field trial Manuel Da Costa*, Kalen Cookson, Jon Schaeffer *Zoetis - U.S. Poultry*

Broiler live-side *Salmonella* spp. interventions have been recognized as an important component for reducing the *Salmonella* numbers coming into the processing plants. Live *Salmonella* Typhimurium vaccines (LVST) are one of the key live-side tools that are part of successful *Salmonella* spp. control programs reducing the risk of *Salmonella* spp. prevalence in poultry final products. The objective of this trial was to compare two LVST programs on *Salmonella* spp. prevalence in commercial broiler chickens. Study Design: there were two LVST vaccination programs composed of either vaccine A or vaccine B assigned to a broiler complex (spray at the hatchery followed by a field boost at 2 weeks of age). This was a 12-week trial with two replicate blocks of two weeks of vaccine B sandwiched in between two weeks of vaccine A (2 weeks before and 2 weeks after). Performance of the LVST programs was evaluated by taking daily hot carcass rinsate samples (3 samples x 2 flocks/day) and processing them for *Salmonella* spp. enumeration (MPN) and prevalence (enrichment). Results: Overall, LVST B resulted in 34% less *Salmonella* spp. positive rinsates when compared with LVST A (16.2% vs. 24.5% positives, respectively). On both replicate blocks, LVST B had lower incidence of positives. When looking at the number of *Salmonella* spp. positive samples per flock, LVST A had 61.04%, 15.58%, 12.99% and 10.39% of flocks with 0, 1, 2 and 3 positive carcasses, respectively, whereas LVST B had 70.59%, 14.71%, 11.76% and 2.94%. The enumeration results were similar between the two LVST treatments (LVST A = 3.16 log vs LVST B = 3.10 log). Discussion: LVST vaccination is a valuable strategy for risk mitigation of *Salmonella* spp. positive at the processing plant. This trial showed that LVST B program resulted in lower *Salmonella* spp. numbers when compared with LVST A. Therefore, differences between LVST programs can potentially be measured in the field to determine maximum risk reduction.

Key Words: Salmonella, Vaccination, Broilers, Food Safety

P258 Two methods of Escherichia coli challenge to evaluate prevention of airsacculitis Virginia Baxter*, Michelle Carrier, Charles Hofacre *Southern Poultry Research Group*

Escherichia coli is considered the most significant bacterial pathogen of broiler chickens. Cellulitis, septicemia, and airsacculitis in poultry are all caused by *E. coli*. Airsacculitis can lead to mortality and carcass condemnation in broilers at processing plants. Two challenge methods were used to demonstrate *E. coli* challenge and evaluate the methods of airsacculitis prevention. In the first method, intratracheal gavage, a cannula attached to a syringe is used to directly inoculate *E. coli* into the trachea. The second method is placing birds on reused litter, spraying with NDV and IBV vaccine, and spraying with an avian pathogenic *E. coli* (APEC) challenge strain at the peak of viral vaccine reaction.

In a study using the first method there were three treatment groups. Treatment groups 1 and 2 were challenged intratracheally with an APEC challenge strain at 1×10^7 and 1×10^8 CFU/dose. Treatment 3 was the negative control. Each day after challenge five birds were humanely euthanized and necropsied for scoring colibacillosis lesions and collection of samples for *E. coli* isolation. This study was used to determine the best sampling time for isolation of *E. coli* post challenge.

In a study using the second method, birds were placed on reused litter at 21 days with elevated ammonia levels, sprayed with NDV (Lasota) and IBV (Arkansas) vaccines at 25 days, and sprayed with an APEC challenge strain at the peak of viral vaccine reaction at 29 days. This study contained five treatment groups containing two experimental vaccines, reference vaccine, placebo, and no challenge control. On days 31 and 35 half of the birds in each group were scored for airsacculitis lesions. This study was used to determine if the experimental vaccines were effective at providing protection against colibacillosis and prevention of airsacculitis.

Key Words: *E. coli*, Airsacculitis, Cellulitis, Intratracheal, Septicemia

P259 Reduction in *Clostridium perfringens* and *Escherichia coli* levels in litter following treatment with a spray-on, *Bacillus*-based litter amendment product Jodi Delago*, Zachary Zawada, Mueez Ahmad, Shannon Burasco, Thomas Rehberger, Alexandra Smith *Arm & Hammer Animal & Food Production*

Litter management has become increasingly important as No Antibiotics Ever (NAE) production has increased. Used litter is often regarded as an inert bed of wood shavings or rice hulls and manure but is actually a living microbiological environment. Poultry litter, rich in nutrients and moisture, is an ideal environment for microbes. Bacteria, fungi, yeast, and protozoa, as well as insects and nematodes, thrive in litter, with levels fluctuating as they compete for nutrients and available moisture. The constant exposure that birds face as they feed, dust, and bed on this material is significant. Over time, the negative effects of this constant and low-level challenge become measurable. A field trial was conducted to determine the efficacy of a spray-on, *Bacillus*-based litter treatment as an intervention to reduce levels of select poultry pathogens in litter. Broiler houses (n=74) from 26 farms with a history of necrotic enteritis (NE) were selected. The product was applied to the litter surface 1-2 days prior to bird placement. Baseline litter samples, sampled within 3 days of prior-flock harvest, and post-treatment litter samples, sampled within 3 days of post-treatment flock harvest, were taken from each house at the waterline. Aerobic bacteria, clostridia, *Clostridium perfringens*, *Escherichia coli*, and non-*E. coli* coliforms were enumerated using standard plate count methods. Overall, levels of each bacterial group decreased after treatment with the product, with all houses seeing a reduction in at least one bacterial group. Clostridia decreased from 4.6 to 4.2 log₁₀ CFU/g (p=0.002), while *C. perfringens* decreased from 3.9 log₁₀ CFU/g to 2.9 log₁₀ CFU/g (p<0.0001) post-treatment. *E. coli* dropped from 4.4 to 3.7 log₁₀ CFU/g (p<0.0001), while non-*E. coli* coliform levels also declined from 3.8 to 3.4 log₁₀ CFU/g (p=0.005). Total aerobic bacteria fell from 8.8 to 8.3 log₁₀ CFU/g (p<0.0001) following treatment. The reduction of potential poultry pathogens in litter indicates that this product may have an impact on broiler health and performance by reducing exposure to pathogens. All houses used in the trial had a history of NE disease issues and lowering *C. perfringens* levels in the environment could reduce further outbreaks in these houses.

Key Words: *Clostridium perfringens*, *Bacillus* probiotic, litter amendment

P260 Application of 16S rRNA sequencing technology to assess broiler microbiome robustness in commercial field analyses John Schleifer*, Tomasz Cieplak, Dorte Sandvang, Jean-Christophe Bodin, Stephanie Frankenbach, Christophe Bostvironnois *Chr. Hansen A/S*

The microbiota of the broiler gastrointestinal tract is complex yet considered the most important anatomical component of general broiler production. A rapid and effective technology to assess the microbiome composition and use it as a predictive model toward broiler production improvements has exceptional merit. A 16S rRNA sequencing technology has been developed to rapidly and accurately analyze poultry microbiome, both qualitatively and quantitatively, utilizing an Oxford Nanopore technology. A Robustness Index has been developed to simplify complex microbiome results. This index is calibrated to accurately reflect microbiota differences for comparison purposes between diets, farms, and operations. Reported here are the results of a proof-of-concept trial utilizing the 16S rRNA sequencing technology, analyzing the microbiome of broiler ceca and employing a Robustness Index. Over a period of seven months commercial broilers from 14 farms from four different geographic locations in the United States were sampled. Sampling ages ranged from d16 to d30. Farms were selected to diversify expected production performances. Bird ages were identified to minimize microbiota interference with feed ration changes. Ceca were harvested and rapidly chilled; followed by DNA extraction and 16S rRNA sequencing at a central laboratory. Robustness Indexes ranged from 59 to 80 and appear to correlate within geographic locations. Variations in microbiota diversity were observed between farms. The genera in most abundance from the sampling sets are *Faecalibacterium*, *Lactobacillus*, *Christensenellaceae* R-7, *Ruminococcus* and *Bacteroides*. The 16S rRNA sequencing technology shows promise as a reliable and effective indicator of broiler microbiome populations. The Robustness Index shows promise as a reliable indicator of broiler intestinal health utilizing microbiome diversity, uniformity, and taxonomical composition. Additional research is needed to correlate predictive performance results and the indices achieved with the 16S rRNA sequencing.

Key Words: Microbiome, broiler, gastro-intestine, 16S rRNA

P261 Compatibility of a recombinant HVT-IBD vaccine with CVI to provide protection against very virulent Marek's and very virulent IBDV challenge in SPF birds Amy Brown, Lauren Taylor*, Megan Bosserd, John Dickson, Jennifer Embrey, Angela Hartman *Zoetis*

Due to the increasing presence of circulating very virulent (vv) and vv+ Marek's isolates in the field, the combination of HVT vectored vaccines such as HVT-IBD with a Rispens vaccine is critical to provide protection especially in long lived layers and breeders. This study assessed the combination of HVT-IBD with a CVI988 Rispens vaccine either in ovo or subcutaneous at hatch on the protection provided against a Day 5 vvMDV RB1B challenge and a Day 14 vvIBD challenge in SPFs. Compatibility was demonstrated for vvIBD and showed 75% efficacy following subcutaneous administration and 80% efficacy following in ovo injection. Compared to previous studies utilizing the HVT-IBD vaccine alone, that have shown 90-93% efficacy against vvIBD, there may have been a small degree of temporary interference, which may have related to the Day 14 challenge and early competition of HVT-IBD and Rispens vaccines to colonize the target cells, T lymphocytes. However, the clinical protection against a very virulent IBD challenge was still very strong. The combined vaccines showed 96% and 98% protection against vvMDV following in ovo and subcutaneous at hatch administration, respectively. Thus, the combination of HVT-IBD with a CVI-988 vaccine provides a strong compatible solution for protecting against circulating field challenges.

Key Words: recombinant, HVT-IBD, CVI, Marek's disease, infectious bursal disease

P262 A systematic review of pathobiological studies of Goose-Guangdong lineage of high pathogenicity H5 influenza viruses in various avian species Christina Leyson*, Sungsu Youk, Mary Pantin-Jackwood *Southeast Poultry Research Laboratory, United States National Poultry Research Center, Agricultural Research Service, United States Department of Agriculture*

Avian influenza viruses (AIV) are a diverse group of viruses that have a wide host range. A particular lineage of highly pathogenic H5 subtype AIVs called the Goose-Guangdong lineage (Gs/Gd) has spread across the globe since it was first reported in 1996. Gs/Gd H5 AIVs continue to frequently cause outbreaks in both domestic poultry and wild bird species and thus, the Gs/Gd H5 viruses has had significant impact on poultry production. For example, it is estimated that 7.5 million turkeys and 42 million chickens died or euthanized during the 2014-2015 Gs/Gd H5 virus outbreak in the United States alone. The understanding of the pathogenesis, infectivity, and transmission of Gs/Gd H5 viruses is important in preventing such significant impact on animal health and on the economy. There is an abundance and long history for data on experimental AIV in-

fections in avian species. To our knowledge, there is no existing database available that has systematically put together experimental information such as virus infectious dose and transmissibility from multiple studies. The goal of this review is to systematically compile experimental information on pathobiological studies of Gs/Gd H5 viruses in avian species in a database and to provide an overview of prevailing findings. As expected, gallinaceous species infected with Gs/Gd H5 viruses have severe disease and eventually succumb to infection, albeit requiring a relatively higher infectious dose. On the other hand, waterfowl species infected with Gs/Gd H5 viruses require a lower dose to become infected and have a wide variety of disease outcomes from mild clinical signs to severe fatal disease. This systematic review of pathobiological studies will be able to provide insights on the pathogenesis and epidemiology of AIVs in avian species, which is important for preventing and mitigating future outbreaks.

Key Words: avian influenza, pathobiology, pathogenesis, Goose-Guangdong lineage, H5 subtype

Environment, Management and Animal Well-Being: Environmental Impacts

P263 Effects of Cyclic Chronic Heat Stress on the Expression of Nutrient Transporters in the Jejunum of Modern Broilers and Their Ancestor Wild Jungle Fowl Alison Ramser^{2,3}, Nedra Abdelli¹, Elizabeth Greene², Lesleigh Beer², Travis Tabler², Sara Orlowski², Jose Perez¹, David Solà-Oriol¹, Nicholas Anthony², Sami Dridi^{2,3} *¹Universitat Autònoma de Barcelona, Department of Animal and Food Sciences, ²University of Arkansas, Center of Excellence for Poultry Science, ³University of Arkansas, Cell and Molecular Biology*

Heat stress (HS) has been reported to disrupt nutrient digestion and absorption in broilers. These effects may be more prominent in fast-growing chickens due to their high metabolic activity, but the underlying molecular mechanisms are not yet fully elucidated. The current study aimed to evaluate the effect of chronic HS on jejunal nutrient transporters in slow- (Athens Canadian Random Bred, ACRB from 1950), moderate- (1995 random bred, 95RAN), rapid- (modern random bred, MRB) growing birds and their ancestor jungle fowl (JF). 150 Male chicks per line were placed by line in environmentally controlled chambers and kept under industry-standard conditions until d28. On d29, an 8-h daily cyclic HS (36°C) was applied to half the chambers, until d55, keeping the rest at thermal neutral (TN, 24°C) conditions. Jejunum tissues were collected for morphology assessment and molecular analysis of carbohydrate-, amino acid-, and fatty acid-transporters. MRB had the highest body weight (BW) followed by 95RAN under both conditions. HS decreased feed intake (FI) in MRB and 95RAN, resulting in lower BW compared to TN counterparts. No effect was observed in ACRB and JF. MRB had greater villus height (VH) to crypt depth (CD) ratio under both environments. Molecular analyses showed that glucose transporter (GLUT) 2, 5, 10, and 11 were upregulated in MRB compared to other populations in TN. HS downregulated GLUT2, 10, 11, and 12 in MRB while it increased the expression of GLUT1, 5, 10, and 11 in JF. GLUT2 protein expression was higher in JF compared to ACRB and MRB in TN. It was increased in ACRB and no effect on 95RAN and MRB under HS. ACRB had greater expression of the EAAT3 gene as compared to the rest of the populations maintained in TN. HS exposure did not alter the gene expression of amino acid transporters in MRB. Gene expression of CD36 and FABP2 was upregulated in HS JF. Protein expression of CD36 was downregulated in HS JF while no effect was observed in ACRB, 95RAN, and MRB. Taken together, these data are the first to show the effect of HS on jejunal expression of nutrient transporters in three broiler populations representing 70 years of genetic

progress in the poultry industry and a Red Jungle Fowl population representative of the primary ancestor of domestic chickens.

Key Words: heat stress, glucose transporters, amino acid transporters, fatty acid transporters, broilers

P264 The effect of *Spirulina platensis* inclusion on live performance and meat quality of commercial broilers subjected to cyclic heat stress Kirsten Shafer^{GS}, Garrett Mullenix, Maricela Maqueda, Travis Tabler, Kentu Lassiter, Walter Bottje, Michael Kidd, Sara Orlowski *University of Arkansas*

Broiler growth and performance can be affected by a multitude of factors including genetics, management, and environment. With rising global temperatures, potential for long bouts of heat stress may become a potential issue when managing broilers for peak performance. Finding ways to alleviate the negative effects of heat stress on broiler performance is imperative. The current study aimed to investigate the effects of *Spirulina platensis* freshwater algae inclusion on the live performance and processing characteristics of commercial broilers subjected to daily cyclic heat stress. Day old Ross 708 male broilers were placed into 8 environmentally controlled chambers (n=30 birds/chamber). Broilers were fed a common starter diet from d 0 to d21 and were reared under standard conditions. At d21, four chambers remained on a control diet containing no algae (CON) while 4 chambers were given a diet that included algae at an inclusion rate of 2.5% (ALG). An 8 hour daily cyclic heat stress (24°C to 36°C) was applied to the chambers from d22 to processing at d48. Daily feed and water intake were recorded as well as weekly body weights. Broilers were processed at d48 and subjected to a full cut up and meat quality evaluation. The data were analyzed using JMP 16.0 with a significance level set at P<0.05. No differences were observed for FCR, WCR or live weight throughout the grow-out period. Additionally, there was no significant difference in mortality between the treatments (ALG=5.8%, CON=3%). At processing, no differences were observed between the groups for carcass weight, wing yield, breast yield, tender yield, and leg quarter yield, breast pH, breast L* or drip loss. The ALG birds had a higher average woody breast score (P=0.0366), a* value (P<0.0001), b* value (P<0.0001) than the CON birds. Inclusion of *Spirulina platensis* algae at an inclusion level of 2.5% did not appear to have much effect on live performance and meat quality of broilers subjected to cyclic heat stress.

Key Words: Heat Stress, *Spirulina platensis*, Broiler, Meat Quality, live production

P265 Effects of alum and acidified bamboo biochar on nitrogen loss from broiler litter during storage S.K.P.M Siriwardana, N.S.B.M Atapattu* *Department of Animal Science, Faculty of Agriculture, University of Ruhuna*

Loss of nitrogen (N), mainly as ammonia (NH_3), from poultry litter causes negative economic, environmental and social impacts. Alum (AL) controls NH_3 losses by reducing litter pH. High porosity and adsorption capacity make biochar a potential amendment for reducing NH_3 losses from poultry litter. This study evaluated the effect of AL and acidified bamboo biochar (ABC) mixtures on N losses from broiler litter during storage. Rice-hull litter on which broilers had been raised for 40 days was windrowed for two weeks and then used for the experiment. The pH of the biochar was reduced from 11.2 to 3.2 by submerging it in water for 2 days, washing and draining and, subsequent immersion in 5% citric acid solution for 2 days. The experiment followed a completely randomized design. Plastic pots (n=49) were filled with 785 g of litter/pot and one of the seven amendment mixtures (15 g/pot) thus giving following treatments; 1) 15% sand (control), 2) 15% ABC, 3) 12% ABC+ 3% AL, 4) 9%ABC+6% AL, 5) 6%ABC + 9% AL, 6) 3% ABC + 12% AL and 7) 15% AL. Pots were incubated from 21 days. Litter samples taken from each pot on day 1 and 21 were analyzed for pH, moisture, electrical conductivity and N. At day 1 the pH of the litter treated with 15% AL (4.2) was significantly ($p<0.001$) lower than that of 15% sand (7.8) and 15% ABC (7.7). Reducing the percent AL from 15 to 0% with a concomitant increase in ABC resulted in significantly higher initial pH values. The 15% ABC treatment resulted in significantly lower pH on day 21 compared to 15% sand treatment. Compared to all other treatments, 15% AL resulted in significantly lower pH on day 21. Increasing ABC from 3% to 12% in mixture with concomitant reduction of AL had no significant effect on litter pH on day 21. Nitrogen losses from litter treated with 15% AL (55%) and 3% AL + 12% ABC (53.3%) were significantly ($p<0.05$) lower than that of 15% sand (63%). Nitrogen losses from litter treated with 12% AL + 3% ABC (42%) and 9% AL + 6% ABC (38.7%) were significantly lower than those from all other amendment combinations. AL 3% + ABC 12% combination was also reported lower N loss (53.3%) compared to 15% sand. The study concludes that AL and ABC mixtures, even one with as low as 3% AL are effective in reducing N losses from broiler litter.

Key Words: Alum, Ammonia, Biochar, Amendment, Poultry

P267 Evaluation of antimicrobial and heavy-metal resistance patterns of avian pathogenic *Escherichia coli* associated with broiler breeder colibacillosis in Mississippi Jiddu Joseph*^{GS}, Madalyn Jennings, Li Zhang, Pratima Adhikari, Reshma Ramachandran *Mississippi State University*

Avian Pathogenic *Escherichia coli* (APEC) is the organism causing colibacillosis in poultry, a leading cause of poultry mortality worldwide. Controlling the disease among broiler breeders is crucial because they are responsible for vertical transmission to progeny. To control this disease, it is important to monitor the antimicrobial and heavy metal resistance patterns among APEC isolates causing colibacillosis. In this study, we analyzed the resistance patterns of 28 APEC strains isolated from broiler breeders with colibacillosis obtained from Mississippi Poultry Research and Diagnostic Laboratory. *E. coli* were isolated in MacConkey agar and DNA was extracted for PCR detection of 11 antimicrobial and 11 heavy metal resistance genes. All isolates were subjected to antibiotic susceptibility testing against 10 antibiotics by Kirby-Bauer disk diffusion method and copper susceptibility testing by broth microdilution assay. Results showed that 93% of the isolates carried at least one antimicrobial resistance gene, with genes conferring resistance to tetracyclines *tetA* (68%) showing the highest prevalence, followed by aminoglycosides *aph (3')-Ia* (50%) and *aadA* (25%), and quaternary ammonium compounds *qacEA* (21.40%). The genes conferring resistance to β -lactamase inhibitors *bla-TEM* (17.90%), cephalosporins *blaCTX-M* (14.30%), and sulfonamides *sulI* (14.30%) were also identified. Among the isolates 10.70% exhibited multidrug-resistance with highest resistance towards tetracycline (28.60%), streptomycin (18%), and kanamycin (14.3%). Furthermore, all isolates carried at least one heavy metal resistance gene with highest prevalence for arsenic-encoding gene *arsC* (100%). The genes-encoding for silver *silE* (35.71%), copper *pcoD* (25%), tellurite *terF* (21.43%), and mercury *merA* (10.71%) were also identified in these isolates. All isolates were susceptible to copper with a minimum inhibitory concentration of 1024 $\mu\text{g/ml}$. In conclusion, this study exhibits high prevalence of antimicrobial and heavy metal resistance genes as well as multi-drug resistance among APEC from broiler breeders. Our findings provide an insight into the patterns of APEC antimicrobial and heavy metal resistance, especially in broiler breeders, as they play an important role in the transmission of APEC.

Key Words: *Escherichia coli*, colibacillosis, broiler breeder, antimicrobial resistance, heavy metal resistance

P270 Survey of electrical grounding systems for commercial broiler houses in Mississippi and Alabama Matthew Rowland^{*1GS}, Daniel Chesser¹, John Linhoss², Joseph Purswell³, Jesse Campbell², Jeremiah Davis² ¹Mississippi State University, ²Auburn University, ³USDA Agricultural Research Service - Poultry Research Unit

A field survey was conducted on fourteen commercial broiler farms in Mississippi and Alabama to evaluate the resistance values of electrical grounding systems on commercial broiler operations. Ninety-six commercial broiler houses constructed between 1989 and 2021 were surveyed. Earth ground resistance values (ohms) of control room service panels, generators, and/or other electrically grounded equipment were measured. House age and grounding system type (traditional ground rod or Ufer) was also recorded. Electrical resistance ratings were measured using an earth ground resistance meter. Differences in mean resistances between Ufer grounding systems vs. traditional grounding rod electrodes were analyzed using a mixed model in SAS. Means were adjusted using house age as a covariant to account for differences in construction practices and significance was considered at $P \leq 0.05$. 40.6% of surveyed broiler houses had a Ufer grounding system, most of which were constructed between 2007 and 2021. 63.5% of surveyed broiler houses (32 traditional vs 29 Ufer) were at or below the 25 ohms resistance value recommended by the National Electric Code (NEC). Ufer grounding resulted in lower resistance ratings (32.54 ± 20.474 ohms) than traditional grounding rods (59.99 ± 16.607 ohms). Ufer grounds are becoming more commonplace in broiler house construction, and they are helping reduce earth ground resistances, however, producers should still inspect their grounding systems annually to mitigate lightning-induced damage. Results from this survey illustrate there is potential for many broiler houses to have a catastrophic loss due to elevated ground resistance.

Key Words: Electrical resistance, Electrical grounding, Ufer

P271 Evaluating the effect of circulation fans on broiler footpad dermatitis severity in commercial houses Zoie McMillian^{*1GS}, Anna Magnaterra¹, Ashlyn Snyder¹, Gregory Martin², Jonathan Moyle³, Shawna Weimer¹ ¹University of Maryland, College Park, ²Penn State Extension, ³University of Maryland Extension

Footpad dermatitis (FPD) is a contact dermatitis on the ventral footpads that is a threat to broiler health and welfare and causes painful lesions if severe. Ventilation is an important management factor in broiler production systems and additional air movement in commercial houses could positively affect the welfare of broilers by reducing footpad dermatitis severity and stabilizing environmental conditions. The objective of this study was to evaluate the effect of circulation fans on the footpad dermatitis prevalence and severity of commercial broilers. We hypothesized that broilers raised in houses with circulation fans would have less severe footpad dermatitis than broilers raised in houses without circulation fans. This study was conducted in 4 commercial organic broiler houses in eastern Maryland. Two, 60 x 600 ft. houses had 16 high-capacity circulation fans (Houses 1 and 2) and two did not (Houses 3 and 4). Each house was divided into 4 equal quadrants for welfare assessments at 3 and 6 weeks of age. During the welfare assessments in each house, 20 birds within each quadrant (N=80 birds/house) were scored for footpad dermatitis on a 0 to 2 scale where 0 indicated no evidence of FPD and 2 indicated severe FPD. Digital images were taken of each bird's feet and the lesion surface area (%) of birds with a score of 1 or 2 were measured in ImageJ. Right and left feet were averaged for analysis. The effects of house and fan treatment on footpad score data was analyzed with chi-square tests. Lesion surface area data was analyzed with t-tests and one-way ANOVAs. Out of all the birds with FPD (score of 1 or 2), birds in the houses without the circulation fans

had greater ($P = 0.0004$) FPD severity scores. Birds in the houses without the circulation fans also had greater ($P < 0.001$) lesion areas than birds in houses with fans (6.8% vs. 4.2%). Birds in House 1 had smaller ($P \leq 0.009$) FPD lesion areas (3.1%) than birds in the other houses (7.2-5.5%). Image analysis of lesions on footpads has the potential to be a specific method of evaluating FPD severity in broilers. The results of this study suggest that the addition of circulation fans in commercial broiler houses could aid in decreasing FPD severity.

Key Words: welfare, broilers, footpad dermatitis, circulation fans

P272 Assessment of water meter accuracy in commercial broiler houses Evan Johnson^{*IUG}, Jeremiah Davis¹, Jesse Campbell¹, Joseph Purswell², Martha Rueda¹, Carson Edge¹, Kelly Griggs¹, Cody Smith¹
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A field survey was conducted on seven commercial broiler farms in South Alabama to evaluate the accuracy of water meters used in the integrated house controller (IHC). A total of 50 displacement type water meters were evaluated across twenty-five 60 x 500 ft commercial broiler houses, ranging in age from new (never used) to eight years old, each having two water meters (front and back) providing bird water consumption input to the IHC. Two portable calibration carts were constructed with two water calibration systems each. Water meters were removed from the house water manifold and compared to a standard water meter (M25, Badger) at three flow rates: 1, 5, and 15 gpm, using a modified method from AWWA Manual of Water Supply Practices M6. It took approximately 1.5 man-hours to evaluate both water meters in each house using the calibration carts. Meter accuracy was determined for each water meter and compared at the $\pm 10\%$, $\pm 5\%$, and $\pm 1.5\%$ levels. The $\pm 1.5\%$ level is required for revenue-grade metering. The AWWA requires meters that fall outside $\pm 10\%$ accuracy must be repaired or replaced. The economical displacement type meters used in these broiler houses had rated flow rates of 1 to 20 gpm with stated transitional and nominal flow accuracies of $\pm 5\%$ and $\pm 2\%$, respectively. Water meters that fell outside the $\pm 10\%$, $\pm 5\%$, and $\pm 1.5\%$ accuracy thresholds were 28% (14/50), 70% (35/50), and 94% (47/50), respectively. The mean accuracy across all meters was 12.6%, 3.1%, and 2.3% for the flow rates of 1, 5, and 15 gpm, respectively. Fourteen meters needed to be evaluated for repair or replacement based on the 10% accuracy threshold. If we remove these 14 meters, the mean accuracy reduced to 4.0%, 2.1%, and 1.7% for the flow rates of 1, 5, and 15 gpm, respectively. This study has shown that if water meter data is to be sent to the cloud for analysis, interpretation, and decision management, water meter accuracy standards must be developed and water meter accuracy must be validated and calibrated if necessary. Expertise would have to be developed to properly operate the test stands and perform calibrations.

Key Words: water flow rate, water consumption, water meter, accuracy, controller

P273 Survey of gas line leaks in commercial broiler houses Baylor Arnold^{*IUG}, Jeremiah Davis¹, Jesse Campbell¹, Joseph Purswell², Carson Edge¹, Martha Rueda¹, Kelly Griggs¹, Cody Smith¹
¹National Poultry Technology Center at Auburn University; ²USDA ARS Poultry Research Unit

A field survey was conducted on eight commercial broiler farms in South Alabama to evaluate the presence of gas leaks on the high pressure (10 psi) trunk line. Gas trunk lines usually run on the outside of poultry houses exposing them to temperature swings that cause expansion and contraction of the steel piping. Each threaded connection has the potential to leak. Of the twenty-seven 60 x 500 ft houses, eight were (NEW; never used) and 19 ranged in age from 5 to 8 years (OLD). The total number of connections between the storage tank/meter and the low-pressure step-down regulator ranged from 88 to 126. A soap solution in a hand sprayer was used to spray each connection and evaluate the presence of a leak through

bubble formation. It took approximately 0.5 man-hours to evaluate the high-pressure piping on each house. The number of leaks was evaluated as a percentage of total connections. Percentage data were arcsin transformed prior to analysis as a one-way (age) ANOVA with the MIXED procedure in SAS. MS were separated at $P \leq 0.05$ with the PDIFF option. House age did not significantly ($P = 0.066$) affect mean number of leaks (NEW = $0.6 \pm 0.2\%$ vs. OLD = $3.1 \pm 0.1\%$). Six of the eight NEW houses had a single leak; one of which was large enough to be a safety hazard. From the OLD farms, three farms (10 houses) had a mean of 8.4 ± 1.4 leaks per house while the three other farms (9 houses) had a mean of 1.2 ± 1.9 leaks per house. From these results, it appears that leaks happen to be more about craftsmanship and/or thread compound quality than house age. Poultry producers should get written verification from the installer that newly constructed houses are free of leaks before operating the farm. It would be useful for poultry producers to check for leaks every few years to verify system integrity.

Key Words: heating fuel, natural gas, propane, gas leaks, broiler house

P274 Assessment of static pressure accuracy in commercial broiler houses Juliana Rezek^{*IUG}, Jeremiah Davis¹, Jesse Campbell¹, Joseph Purswell², Carson Edge¹, Kelly Griggs¹, Martha Rueda¹, Cody Smith¹
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A field survey was conducted on seven commercial broiler farms in South Alabama to evaluate the accuracy of static pressure (SP) sensors used in the integrated house controller (IHC). A total of 25 SP sensors were evaluated across twenty-five 60 x 500 ft commercial broiler houses, ranging in age from new (never used) to eight years old, each having one SP sensor providing input into the IHC. Static pressure measured by each IHC pressure sensor was compared to a high-resolution SP meter (922, Fluke) at target levels of 0.05, 0.10, 0.15, and 0.20-inch WC to encompass the range expected during production. The low-pressure tube for the IHC pressure sensor was already installed through the house wall while the Fluke low pressure tube was inserted through a crack in the control room door to avoid drilling access holes through the wall. It took approximately 0.5 man-hours to evaluate the SP sensor in each house. Differences between IHC pressure sensors and the standard meter were analyzed as a one-way (levels) ANOVA using the MIXED procedure in SAS. MS were separated at $P \leq 0.05$ with the PDIFF option. Pressure level did not significantly ($P = 0.96$) affect mean difference in SP between the IHC pressure sensors and the standard. IHC pressure sensors measured SP an average of -0.01 inch WC lower than the standard meter with differences ranging from -0.05 to 0.01 inch WC.

Key Words: static pressure, pressure meter, pressure sensor, accuracy, controller

P275 Assessment of temperature sensor accuracy in commercial broiler houses Leah Smith^{*IUG}, Jeremiah Davis¹, Jesse Campbell¹, Joseph Purswell², Carson Edge¹, Martha Rueda¹, Kelly Griggs¹, Smith Cody¹, John Linhoss¹
¹National Poultry Technology Center; ²USDA ARS Poultry Research Unit

A field survey was conducted on six commercial broiler farms in South Alabama to evaluate the accuracy of temperature sensors used in the integrated house controller (IHC). A total of 168 temperature sensors were evaluated across twenty-two 60 x 500 ft commercial broiler houses, ranging in age from new (never used) to eight years old, each having between six and eight temperature sensors providing input into the IHC. Four portable calibration carts were constructed with a small generator. Temperature measured at each IHC sensor was compared to a dry well temperature calibrator (9102S, Fluke) at target temperatures of 55, 70, 85, and 100 °F to encompass the range of expected temperatures during production. It took approximately six to eight man-hours to evaluate all sensors in each house. Differences between IHC temperature sensors and the stan-

dard meter were analyzed as a one-way (target temperature) ANOVA with the MIXED procedure in SAS. Means were separated at $P \leq 0.05$ with the PDIF option. Target temperature significantly ($P < 0.0001$) affected the mean temperature offsets (IHC - std); mean offsets were +2.43, +1.19, -0.29 and -2.05 °F for target temperatures 55, 70, 85, and 100 °F, respectively. Ranges for IHC temperature sensor measurements were 54.9 – 63 °F, 69.9 – 74.2 °F, 82.2 – 86.7 °F, and 94.2 – 100.8 °F for target temperatures 55, 70, 85, and 100 °F, respectively. The IHC sensors were reading low at high target temperatures potentially forcing heaters to run longer than required. IHC sensors were reading high at low target temperatures which could force fans to run earlier and longer than required. If temperature data is to be sent to the cloud for analysis, interpretation, and decision management, IHC temperature sensor accuracy must be validated and calibrated if necessary.

Key Words: indoor temperature, temperature sensor, accuracy, controller

P276 Survey of light uniformity in commercial broiler houses Abigail Lane¹UG, Jeremiah Davis¹, Carson Edge¹, Jesse Campbell¹, Joseph Purswell², Kelly Griggs¹, Martha Rueda¹, Smith Cody¹, John Linhoss¹
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A field survey was conducted on five commercial broiler farms in South Alabama to evaluate the uniformity of light intensity across three locations during brooding and tunnel ventilation modes. Twenty 60 x 500 ft commercial broiler houses, ranging in age from new (never used) to eight years old, had three lines of lights above the feed lines. Brood and non-brood LED light bulbs were installed with an alternating spacing of 8-ft on-center. Target light intensity during tunnel ventilation was 0.2 lux. Seven light intensity sleds were pulled down the long-axis of each house in 40-ft sections in the following house locations: evaporative pads (PAD), at center house (CENTER), and between the tunnel fans (FAN). It took approximately eight man-hrs to evaluate each house. Mean light intensity and CV were calculated across 70 measurements for each location and analyzed as a two-way (age and location) ANOVA using the MIXED procedure in SAS for both brooding and tunnel ventilation modes. Means were separated at $P \leq 0.05$ with the PDIF option. During brooding, mean light intensity was different for age ($P < 0.0001$) with NEW (86.6 lux) having higher intensities than OLD (20.0 lux), mean CV was different for age ($P = 0.0004$) with NEW (24.6%) having better uniformity than OLD (36.7%), mean light intensity was not different for location ($P=0.945$) at PAD (53.6 lux) and CENTER (53.0 lux), and mean CV was not different for location ($P = 0.1675$) at PAD (32.6%) and CENTER (28.7%). During tunnel ventilation; mean intensities at PAD and CENTER were lower ($P < 0.0001$) than FAN for OLD (0.05, 0.06, and 77.4 lux, respectively) and NEW (0.32, 0.30, and 74.7 lux, respectively), PAD and CENTER in NEW houses were slightly higher than target (0.2 lux) while PAD and CENTER in OLD houses were much lower than target where many had areas with undetectable light intensities, and mean CV was different for both age ($P < 0.0001$) and interaction between age and location ($P = 0.01$). Light uniformity in tunnel ventilation was four to six times better in the PAD and CENTER areas of the NEW (36.5% and 43.6%, respectively) compared to OLD (228.3% and 191.7%, respectively). Uniformity in FAN area was 149.5% vs 123.0% for OLD and NEW, respectively.

Key Words: light intensity, light uniformity, light measurement, broilers

P277 Impact of eggshell disinfection on the bacterial load and microbiome of broiler hatching eggs John Linhoss¹, Maryam Mohammadi-Aragh², Evans Jeff³ ¹National Poultry Technology Center, ²Mississippi State University Agricultural & Biological Engineering Department, ³USDA ARS Poultry Research Unit

Reducing bacterial loads in broiler eggs entering the hatchery is critical for biosecurity and food safety programs. Exploration of various disinfectants and application strategies to mitigate bacterial loads on hatching eggs is needed, especially as market demand for antibiotic-free poultry increases.

200 eggs from a 50-week-old flock of Ross 708 broilers were randomly assigned to the following disinfection treatments: BioShield 75®, Virocid®, 150 ppm sodium hypochlorite (bleach), water, and a dry control. 40 eggs per treatment were disinfected using an electrostatic sprayer. Each egg was individually rinsed with PBS, and the rinsates were evaluated for bacterial cfus using Aerobic Count Plate petrifilms (3M). Bacterial counts were measured 1 and 3 h post-disinfection (20 eggs per sampling time) and treatment reps were pooled for 16S rRNA sequencing analysis. Main effects of sampling time and disinfectant significantly affected eggshell bacteria counts ($P < 0.0001$). Disinfectant* sampling time interaction effects were also significant ($P = 0.0302$). BioShield® 75, Virocid®, and sodium hypochlorite led to significant overall reductions in microbial contamination when compared to the water and dry controls. Virocid® application resulted in the lowest overall bacterial load and a 2.6_{log10} CFU/egg reduction when compared to the dry control treatment. BioShield® 75 resulted in the second lowest bacterial load and a 2.1_{log10} CFU/egg reduction when compared to the dry control treatment. The water and dry control treatments had the highest bacterial loads and were not significantly different from each other. Average bacterial counts were 10.8% lower at the 1 h sampling time when compared to 3 h sampling time. Alpha diversity, as measured by the Shannon diversity indices, showed that species evenness and richness was significantly affected by disinfection ($P < 0.05$). However, beta diversity, as measured by principal component analysis (PCA), indicated no clear distinction in phylogenetic assemblage amongst treatment groups ($P < 0.05$). Overall, electrostatic application of the three disinfectants tested lowered bacterial loads on broiler hatching eggs.

Key Words: hatching eggs, bacteria, electrostatic spraying, disinfection

P279 Effect of age at photo-stimulation on performance, egg quality, reproductive performance, and skeletal properties of laying hens Amin Rahimi^{1GS}, Prafulla Regmi², Kenneth Anderson¹ ¹*North Carolina State University*, ²*University of Georgia*

The risk of bone fractures is an important welfare concern in commercial egg layer flocks regardless of the housing system. The onset of egg-laying activity in a hen prompts the shift towards medullary bone formation from structural bone tissue. This study aimed to investigate the delay in photo-stimulation on bone quality and egg performance of laying hens. We hypothesized that delaying the onset of egg-laying allows bones to mature and increase their strength. Hy-line W36 and 1940 leghorn pullets reared in floor pens until 9 weeks of age were used in the experiment. Pullets from both genetic lines were then randomly assigned to conventional cages in 2 light-tight rooms. The first room assigned for photo-stimulation at 15 weeks of age (PS15) contained 61 hens, and the other room assigned for photo-stimulation at 20 weeks of age (PS20) and contained 72 hens. Performance (body weight, egg production) egg quality (shell strength, Haugh Units), bone quality (cortical thickness, ash, Ca/P content, break-ing strength) along with plasma parathyroid hormone (PTH), estradiol hormone, and serum calcium and phosphorus were measured at differ-ent points throughout the study. Data were analyzed using mixed-model ANOVA in SAS v 9.4 with $P < 0.05$ considered significant. Tibiae cortical thickness was significantly ($P < 0.05$) higher in 1940 leghorn than Hy-line W36 hens. The anterior, posterior, medial, and lateral dimensions of the tibia cortex from PS15 were significantly ($P < 0.05$) thicker than the tibia cortex of PS20. There was no effect of different ages at photo-stimulation on egg production rate. The Hy-line W36 hens had significantly ($P < 0.05$) better egg production rate, heavier eggs, higher Haugh unit, higher vitel-line strength, and higher shell strength than 1940 leghorn hens. The Hy-line W36 from PS15 had significantly ($P < 0.05$) higher calcium content in their bones than PS20 lighting regimen. The 1940s from PS20 had sig-nificantly ($P < 0.05$) higher calcium and phosphorus content in their bones than PS15 lighting regimen. These results suggest that bones' response to age at photo-stimulation is influenced by their genotype.

Key Words: Photo-stimulation, Parathyroid hormone, Estradiol hormone, Conventional cages, Cortical thickness

P280 Monitoring litter quality in cage-free facilities with W-36 pullets Xiao Yang^{GS}, Ramesh Bist, Sachin Subedi, Yangyang Guo, Casey Ritz, Lilong Chai *Department of Poultry Science, University of Georgia*

Litter quality is critical to birds' health and welfare in both broiler and cage-free laying hen houses. The objective of this study was to monitor litter quality and influential factors for pullets' phase (<17 weeks old) in cage-free houses. In this study, fresh pine shaving at 2.5 cm were used as bedding material for 800 day-old Hy-Line W-36 chicks evenly raised in four identical rooms (denoted as room A, B C, and D, each was 18.6 m²) on the Poultry Research Farm at the University of Georgia (UGA). Air temperature and RH were monitored continuously with HOBO MX2300 Data Loggers. Litter samples were collected from four representative zones (denoted Z1 – close to feeder zone, Z2 - close to nipple drinkers' zone; Z3 – close to walking zone, and Z4 close to perching zone). Litter quality indicators of litter depth, density, mass production, and litter mois-ture content (LMC) were measured weekly. Litter density was estimated by using a 500 mL measure cup and a digital scale; moisture content was measured by drying the litter samples in a 105 °C oven for 24 h; litter gen-eration was estimated based on weekly litter depth, density, and floor area of each room. To quantify the difference in litter quality between rooms or zones in each room and changes over time, the one-way ANOVA and

Tukey HSD analysis were performed by using JMP software. The difference was considered significant at $p < 0.05$. There were no significant differences in weekly LMC, litter depth, volume or production rate between four rooms, but all litter quality indicators were changing over time from week 1 to week 7 (i.e., LMC were $8.58 \pm 0.21\%$ on the week 1, but it reached $10.11 \pm 0.63\%$ on the week 7) ($p < 0.05$). For different zones in the same room, litter depth and LMC were observed with significant difference ($p < 0.05$). The maximum LMC and litter depth were observed on week 7 in the Zone 2 (i.e., drinking area where the LMC was 10.12% and the litter depth was 4.42 cm), while the lowest litter moisture and depth (i.e., 8.9% LMC and 1.83 cm litter depth) were found in the Zone 3 (i.e., walking zone). The research findings will provide reference for commercial cage-free pullets house to monitor and manage litter quality for a better animal wellbeing.

Key Words: cage-free house, pullet, litter quality indicator, animal welfare, litter moisture content

P281 Application of feed additives garlic-cinnamon combination and betaine positively influences production older layers —Colombia commercial study Kim Wilson¹, Lien Vande Maele¹, Jorge Plata², Hector Navarro¹, Diana Hernández¹, Arno van der Aa¹ ¹*Orffa Additives, B.V.*, ²*San Rafael Farm*

Improving production in layers at the end of the cycle can be beneficial both economically and sustainably. Challenges in older flocks include diminished egg quality, reduced feed efficiency, increased susceptibility to disease and overall reduced production[GH1] . The use of feed additives in late production support hens to better deal with the challenges and to maintain health and productivity. Garlic and cinnamon phytochemicals are individually documented as having antimicrobial and anti-inflammatory properties. Betaine contains osmotic properties that support intestinal cellular growth, thus improve feed utilization. Individually or synergistically, these additives are hypothesized to improve egg production. The objective was to evaluate garlic-cinnamon (AP) and betaine hydrochloride (BK) on layer production. Two trials with 2,500 Hy-Line Brown layers each in an open house system (3 birds/cage) with the following treatments[LVM2] : Negative control (CON); Positive control with 120 ppm of Enramycin (AGP[GH3]); CON+125ppm AP (APL); CON+250ppm AP (APH). Trial 1 included APL+500ppm BK (APLBK) and trial 2 APH+500ppm BK (APHBK). Trials went from 64 to 72 weeks. Egg production and egg weights were recorded. A two-way ANOVA was applied and multiple comparisons using Tukey's HSD. Throughout both trials, CON and AGP egg production were statistically similar: in trial 1 72 weeks CON $78.4\% \pm 0.8$; AGP $80.3\% \pm 1.4$; trial 2 CON $77.6\% \pm 0.3$; AGP $78.2\% \pm 0.2$. Trial 1 significance occurred 67-weeks into trial and occurred throughout where APH production ($87.8\% \pm 0.6$) was higher than CON ($80.7\% \pm 1.1$) and AGP ($81.0\% \pm 1.0$) and APLBK ($85.6\% \pm 0.5$) became higher than CON ($81.7\% \pm 1.0$) and AGP ($82.1\% \pm 0.9$) at 68-weeks[LVM4] , $p < 0.05$. Trial 2 APHBK ($85.0\% \pm 0.4$) had elevated production relative to APH ($82.9\% \pm 0.2$) and APL ($81.4\% \pm 0.4$) and APH was elevated compared to CON ($81.1\% \pm 0.2$) and AGP ($81.2\% \pm 0.2$), at 68-weeks, $p < 0.05$. Trial 2 egg weights increased in APH and APHBK relative to CON ($64.4 \text{ g} \pm 0.2$) and AGP ($64.4 \text{ g} \pm 0.1$) final averages at $66.4 \text{ g} \pm 0.4$ and $67.2 \text{ g} \pm 0.6$, respectively, $p < 0.05$. Garlic-cinnamon at 250ppm after 4 weeks positively influenced egg production in older layers, increasing production cycle; betaine may have an additive effect on egg production.

Key Words: layers, garlic, cinnamon, betaine, egg production

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

P282 The effect of geogrid in free-range production systems on the microbiological profile of shell eggs and the environment Lin Walker*, Kenneth Anderson *North Carolina State University*

There are many challenges associated with egg production in the free-range production system which is gaining favor by the consumers and animal welfare groups. However, with this access to the outdoors one of the big concerns is the increased exposure of the hen to pests and pathogens inherent in outdoor environments. A possible means to limit hens access to the soil-borne pests and pathogens while allowing forage growth, is an overlay of a geogrid in the free-range system which is used in Europe and may be gaining interest here. This study compared the free-range system with paddocks covered in geogrid (Insta Turf®) to the conventional grass paddocks in the range system evaluating the microbiological profile of shell eggs and the environment. Two range houses were utilized containing 4 replicates of 60 hens each for a total of 480 hens. Each hen was provided with 1208 cm² of range house and 5.57 m² of paddock space which was divided in half and the hens were rotated every 28 days to maintain a 50% forage cover. Two paddocks in each house were covered in geogrid and 2 were left with standard grass paddocks. Egg samples were collected from each of the replicates at hen ages of 53 (spring) and 65 week (summer), the populations of total aerobic bacteria count, *E. coli* coliform, Enterobacteriaceae, and yeasts & molds from the shell rinse, egg content, and environmental drag swabs were enumerated. The prevalence of *Salmonella* spp. in these three types of samples was also monitored. The data was analyzed using Student t-test ($P < 0.05$). Overall, there was no significant difference ($P > 0.05$) between the geogrid and conventional range samples regarding to each type of bacteria. The only exception was that the population of Enterobacteriaceae from the shell rinse of the eggs collected from the geogrid range was slightly lower (0.5 log₁₀ CFU/mL) than the samples from conventional range at 65-week. However, seasonality was not negligible - a significant ($P < 0.05$) increase of the bacterial population from the environment was observed in summer. The results indicate that the application of geogrid in free-range production systems did not impact on the microbial safety of the shell eggs.

Key Words: free range, geogrid, microbial safety, shell eggs, *Salmonella*

P284 Effect of variable light intensity lighting program on brain activity of commercial broilers Seong Kang^{*1}, Karen Christensen², Michael Kidd Jr.¹, Sara Orłowski¹, James Clark² ¹*University of Arkansas*, ²*Tyson Foods, Inc.*

Environmental light affects behavior and brain plasticity in birds. This study aims to evaluate welfare of birds in the different lighting programs by studying brain activities related with welfare. Day old Cobb 700 chicks were placed in four commercial broiler houses. 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) on a 16L:8D photoperiod. In each section of the house, birds were sampled at 14, 28, and 42 days of age (n=3/section, n=12/house, male). Each bird was sacrificed by cervical dis-location for brain collection, and brains were dissected and snap frozen in dry ice. Two major serotonergic (5-HTergic) regions in brainstem, dorsal raphe nucleus (DRN) and caudal raphe nucleus (CRN), and ventral tegmental area (VTA) were dissected as previously reported for gene expression study of the tryptophan hydroxylase 2 (TPH2: rate-limiting enzyme of 5-HT biosynthesis), tyrosine hydroxylase (TH: rate-limiting enzyme of dopamine biosynthesis), brain-derived neurotrophic factor (BDNF), and glucocorticoid receptor (GR) by qPCR. Differences of gene expression level 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 $p < 0.05$. At the 42 days of age, expression of TPH2 in DRN and VTA of VL birds was significantly lower compared to 5L and 20L birds ($p < 0.05$), but not significantly different with that of NL birds ($p > 0.05$), suggesting the balanced central serotonergic homeostasis in VL treated birds. VTA-TH expression in 5L birds was significantly higher than those of 20L, NL and VL birds ($p < 0.05$), suggesting the high stress-susceptibility of 5L birds. VTA-BDNF expression of NL birds was 2.5 fold higher than those of 5L, 20L and VL birds ($p < 0.05$), and VL birds showed the lowest level of BDNF expression ($p < 0.05$), suggesting the chronic social defeat stress in NL birds compared to 5L, 20L and VL birds. Lower VTA-GR expression in 20L and VL birds indicates low stress in birds compared to NL and 5L birds ($p < 0.05$). Taken together, results of welfare related brain activities indicate the beneficial effects of VL lighting program on broiler welfare in commercial houses.

Key Words: variable light intensity, broilers, welfare, serotonin, BDNF

P285 Ileum transcriptomic analysis of broiler chickens raised in thermoneutral or heat stress condition Ahmed Ghareeb^{*1GS}, Gustavo Schneiders², James Foutz¹, Marie Milfort¹, Alberta Fuller¹, Romdhane Rekaya³, Samuel Aggrey¹ ¹*Department of Poultry Science, University of Georgia*, ²*Merck & Co., Inc.*, ³*Department of Animal and Dairy Science, University of Georgia*

Heat stress (HS) is a seasonal stressor in the poultry industry that impacts health, feed intake, and growth of chickens leading to severe economic losses. The ileum is an intestinal segment where critical processes of nutrient digestion and absorption take place. This study aims to elucidate the transcriptional changes in the ileum tissue in response to HS. One hundred and twenty two-week old male Ross 708 chicks were randomly allocated into two groups in separate rooms: Thermoneutral (TN) group and Heat Stress (HS) group raised at 35°C and 25°C, respectively (Schneiders et al., 2020a,b). Each group consisted of sixty chickens randomly distributed into six cages. Chickens were fed on *ad libitum* unmedicated standard grower diet. On day six of treatment, the ileum tissues of five chickens per group were randomly sampled for RNA extraction and then sequenced using NGS Illumina sequencing platforms. The high-quality reads were processed, and the hit counts were used for identifying the differentially expressed genes (DEGs) for each treatment using DESeq2 at false discovery rate of 0.05. Gene ontology (GO) with GlueGo integrated terms, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway were used for DEGs functional analysis and pathways clustering and identification. A total of 508 DEGs were identified (290 upregulated and 218 downregulated) in the HS group compared with the TN group at fold change ≥ 1.2 ($P < 0.05$). The top-downregulated pathways were oxidative phosphorylation, MAPK signaling, protein processing in the ER, insulin signaling, and apoptosis in the HS group. Multiple immune-, phagocytic-, and lytic-related pathways were also downregulated in the HS group ($P < 0.05$). The top enriched pathways were histidine metabolism, calcium signaling, glycolipid metabolism, and fatty acid degradation. Multiple amino acid metabolic pathways, including histidine, tyrosine, and glycine, serine, and threonine metabolism, were also enriched in the HS group ($P < 0.05$). The cellular molecular system diminishes the immune functions and protein synthesis coincided with a reduction in major energy metabolism in response to reduced nutrient intake. Instead, the heat-stressed chickens relied on multiple amino acids metabolic pathways to maintain their cellular biological needs and antioxidant machinery.

Key Words: Heat stress, ileum, transcriptome

P286 Eimeria maxima and heat stress combined effect on ileum transcriptome profile in meat-type chickens Ahmed Ghareeb^{*1}, Gustavo Schneiders², James Foutz¹, Marie Milfort¹, Alberta Fuller¹, Romdhane Rekaya³, Samuel Aggrey¹ ¹*Department of Poultry Science, University of Georgia*, ²*Merck & Co., Inc.*, ³*Department of Animal and Dairy Science, University of Georgia*

Eimeria (E.) maxima (EM) parasite infects the midgut disrupting the jejunal and ileal mucosal lining causing high morbidity and mortality. Heat stress (HS) is a seasonal stressor that impacts the biological functions leading to poor performance in chickens. Our objective was to elucidate how EM infection alters the gene transcription profile of the ileum tissue under HS. One hundred and twenty 2-week-old males Ross708 were randomly allocated into two groups each of 6 cages and 10 birds per cage. HS control (HS-C) group was received water, and HS-EM group were gavage infected with received 200×10^3 sporulated oocysts/bird, both groups were raised at 35°C (Schneiders et al., 2020a,b). At 6-day post-infection, five chickens per group were randomly sampled, and ileum tissues were collected for RNA extraction and then sequenced using NGS Illumina sequencing platforms. The high-quality reads were processed, and the hit counts were used for identifying the differentially expressed genes (DEGs) for each treatment using DESeq2 at FDR of 0.05. Gene ontology with GlueGo integrated terms, and KEGG pathway were used for DEGs functional analysis and pathways clustering and identification.

Statistical analysis was conducted with a $P < 0.05$ considered significant. A total of 920 upregulated and 988 downregulated were determined in the HS-EM group compared with the HS-C group at cutoff ≥ 1.2 -fold change. Immune-related pathways were highly upregulated in the HS-EM compared with HS-C. Cell cycle, protein processing, DNA and protein repair, cell respiration, arginine metabolism, and junction proteins related pathways were among the enriched pathways. While, the pathways associated with histidine metabolism, calcium signaling, glycolipid metabolism, fatty acid metabolism, and ABC transporters were downregulated. Two prominent stress-related signaling pathways: calcium and PPAR, were potentially downregulated. *E. maxima* infection significantly alters the reported HS-induced changes in the ileum tissue transcriptome profile of the broiler chickens. EM infection overrode the immune-suppressive action of HS. The known HS-induced downregulation of cellular proliferation, metabolism, and protein synthesis was restored by combining EM infection with HS. However, the protein repair and cellular antioxidant capacity associated with HS response are still proceeding in the HS-EM group.

Key Words: Heat stress, *Eimeria maxima*, transcriptome

P287 Influence of chronic and acute heat stress exposure on whole blood gene expression of stress and inflammatory markers in broilers. Jean-Rémi Teysier^{*1GS}, Aurélie Preynat², Pierre Cozannet², Mickaël Briens², Elizabeth Greene¹, Samuel Rochell¹, Sami Dridi¹ ¹*University of Arkansas*, ²*Adisseo France S.A.S.*

Heat stress (HS) is one of the most challenging environmental stressors that decreases bird performance and causes significant economic losses for the poultry industry. The objective of this study was to determine the effects of an acute (aHS) and two chronic HS models, constant (coHS) and cyclic (cyHS), on the expression of stress-related genes in broilers. A total of 720 Cobb male breeder by-product chicks were allocated to 12 environmentally controlled chambers divided into 2 pens of 30 broilers and were reared under thermoneutral conditions until d20. From d20 to d41, 4 chambers were set to 35°C (coHS), and 4 chambers were set to 35°C for 12h and 24°C for the next 12h (cyHS). The 4 remaining chambers were set to a thermoneutral temperature (24°C) with half of the birds pair-fed to equalize FI with coHS birds (TN_{PF}) and the other half fed *ad-libitum* (TN_{AL}). At d41, 16 TN_{AL} birds were randomly selected (4 birds per pen) and exposed to aHS (35°C for 4 hours). Feed intake and BW were measured weekly and blood samples were collected on d41. For the cyHS group, samples were taken before (cyHS₁) and after (cyHS₂) 4h of HS at 35°C. Whole blood gene expression of heat shock proteins (HSP 27, 60, 70, 90), cytokines (IL6, 10, 18, CRP, TNF α), and oxidative stress markers (GPx1, SOD1, SOD2) was determined by RT-qPCR on 8 birds per treatment. Previously reported impacts of HS on performance included an impaired ($P < 0.001$) BWG and FCR from d20 to d41 under coHS and cyHS compared with TN_{AL} condition, and these detriments were greater with coHS than cyHS. Performance of TN_{PF} birds was better than coHS but lower than TN_{AL} broilers. Relative to TN_{AL} birds, gene expression of HSP90 was upregulated ($P < 0.001$) in aHS, cyHS₂, coHS, and TN_{PF} groups, while HSP70 was only increased ($P = 0.027$) in coHS birds. IL-6 expression was higher ($P < 0.001$) in coHS and TN_{PF} birds. SOD1 and SOD2 expression was increased ($P < 0.001$) in aHS, coHS, and TN_{PF} birds. Finally, cyHS₂ birds had increased ($P < 0.001$) HSP90 and GPx1 expression compared to cyHS₁ birds. These data indicate that coHS causes a greater stress response than cyHS and aHS. Also, similar responses between coHS and TN_{PF} birds suggest that some biomarkers evaluated were not specific to HS *per se* but triggered by feed restriction.

Key Words: Heat stress, Biomarkers, Oxidative stress, Inflammation, Broiler

P288 Determining the impact of stocking density on platform usage in fast-growing broilers Cynthia Lopez*, Monica Franco, Katy Tarrant
California State University, Fresno

Environmental enrichments, such as platforms, act to promote natural behaviors in broilers. Enrichments can positively impact broiler welfare, but additional studies are needed to explore usage and implementation in commercial facilities. In an effort to better understand the usage of platforms in fast-growing commercial flocks, this study compared the usage of a platform enrichments in two stocking densities. Eight pens constructed from PVC and chicken wire frames were setup around a water line within a commercial broiler house. Each pen included a hand-fed feeder and a platform measuring 0.5 ft tall with an available sitting area measuring 3 sq.ft. Fast growing broiler chicks were placed in alternating maximum stocking densities of 5.5 lb/sqft (SD55) and 6.5 lb/sqft. (SD65) per bird in each pen. Birds were straight run and provided food and water ad libitum. Platform usage was viewed through cameras affixed to the ceiling at five time points daily (9:30, 13:30, 17:30, and 21:30) from d 7 to d 42. Usage of the platforms was evaluated by the number of birds using the platform and the proportion of birds using platforms, calculated as (# birds using platform / # birds in the pen). Data were compared using a student's t-test in JMP v.16. Average platform use was recorded as being the highest at the 17:30 timepoint (3.77 ± 0.12), significantly larger to only the 21:30 timepoint at 3.28 ± 0.12 ($P = 0.04$). Regardless of stocking density, platform usage was highest in week 2 at an average of 4.5 ± 0.12 , followed by week 6 at 3.96 ± 0.11 ($P = 0.01$). Throughout all days of age, the SD55 pens had the highest proportional use of platforms at 0.156 ± 0.003 , versus 0.131 ± 0.003 for the SD65 pens ($P < 0.0001$). SD55 pens had a significantly higher proportional usage of platforms over SD65 on combined recorded timepoints for week 4, 5, and 6 ($P < 0.001$). The preliminary data gathered in this study represent first steps in exploring how stocking density plays a role in platform usage in a commercial broiler facility. This study will contribute to future studies aimed at identifying best practices in determining platform number based on facility specifics.

Key Words: broiler, platform, enrichment

P289 A preliminary evaluation of the impact of artificial light parameters on Pekin duck activity Gabrielle House*^{GS}, Gregory Archer, Eric Sobotik
Texas A&M University

Two experiments were performed to evaluate the effects of (i) photoperiod duration and (ii) ultraviolet light supplementation on Pekin duck activity during week 3 of grow out. In both experiments, data logger pendants were attached to ducks in each treatment using a harness, and duck activity was logged every 10 s for 48 consecutive h. In Experiment 1, ducks were exposed to one of two photoperiod treatments: 16 h of light, 8 h of dark (16L:8D) or 20 h of light, 4 h of dark (20L:4D). In Experiment 2, ducks were exposed to one of two artificial light treatments: white LED light only (CON) or white LED light with supplemental UV LED light (UV). In both experiments, data logger readings were used to evaluate total duck activity (AT), average photophase activity (PA), average scotophase activity (SA), and differences in treatment means throughout the 48-h analysis period (TOD). Data were analyzed using GLMs, where significant differences were considered when $P < 0.05$. Fisher's LSD post hoc test was used for mean separation. In Experiment 1, PA was elevated in 20L:4D ducks (85%) compared to 16L:8D ducks (82%, $P = 0.004$). Additionally, there was an interaction effect of photoperiod and TOD ($P = 0.039$). There were no differences in AT or SA ($P > 0.05$). In Experiment 2, no differences in TA, SA, or PA were observed, and there were no interaction effects between treatment and TOD ($P > 0.05$). The results of Experiment 1 indicate a 20L:4D photoperiod may stimulate more average activity in Pekin ducks during the photophase period than a 16L:8D photoperiod, although photoperiod does not affect duck total activity or average scotophase activity. The results of Experiment 2 indicate exposing ducks to white LED light with supplementary UV light does not affect total, average photophase, or average scotophase activity compared to white LED light alone. Overall, these two experiments indicate photoperiod is more influential than UV supplementation on 3-wk old Pekin duck activity, and that photophase duration may encourage duck activity at certain times of day.

Key Words: Duck, Behavior, Activity, Lighting, Photoperiod

Metabolism and Nutrition: Amino Acids

P291 Effects of low crude protein diet supplemented with three functional amino acids on the growth performance and oxidative status of broilers challenged with *Eimeria* Spp. Guanchen Liu*^{GS}, Po-Yun Teng, Yuguo Tompkins, Woo Kim
Department of Poultry Science, University of Georgia

This study was aimed to evaluate the effect of low crude protein (CP) diets supplemented with arginine (Arg), threonine (Thr), and glutamine (Gln) separately or in combination on growth performance and oxidative status of broilers challenged with *Eimeria* Spp. A total of 780 14d-old male Cobb 500 broilers were randomly allocated into 10 groups (6 cages/treatment, 13 birds/cage). Nine challenged groups (C) were orally gavaged with 12,500 oocysts of *E. maxima*, 12,500 oocysts of *E. tenella*, and 62,500 oocysts of *E. acervulina*, and one non-challenged control group (NC) was orally

gavaged with PBS. The control diet (20% CP) was fed to NC and one challenged group (CC). Low CP diets (17% CP) with amino acid supplementations were fed to the rest C (T1, No supplementation; T2, 1% Arg; T3, 1% Thr; T4, 1% Gln; T5, 1% Arg+1% Thr; T6, 1% Arg+1% Gln; T7, 1% Gln+1% Thr; T8, 1% Arg+1% Thr+1% Gln). Growth performance was recorded on 6 and 9d post-infection (DPI). The liver was collected on 6 DPI for oxidative status analyses. Data were analyzed by one-way ANOVA and Duncan's multiple range test. At 6 & 9 DPI, feed intake (FI), body weight (BW), and body weight gain (BWG) were higher ($P<0.05$), and feed conversion ratio (FCR) was lower ($P<0.05$) in NC than all C. At 6 DPI, T3, T7, T8 had lower FI, BW, and BWG than other C. T6 had higher ($P<0.05$) BW and T2&T6 had higher BWG and lower FCR ($P<0.05$) than CC. At 9 DPI, FI was higher ($P<0.05$) in CC than all other C except T1. And T8 had lower FI, BW, and BWG than other treatment groups. The lipid oxidation product, malondialdehyde (MDA), in the liver was lower ($P<0.05$) in NC than all C. T8 had a higher ($P<0.05$) MDA content than T1&T6. The activity of superoxide dismutase was higher ($P<0.05$) in T1&T5 than in T3. The activity of glutathione peroxidase was lower ($P<0.05$) in T2&T6 than in CC&T1. The glutathione content was higher ($P<0.05$) in NC than C while no differences were observed among C. In conclusion, under *Eimeria* infection, birds fed a normal diet and low CP diet tended to have the same growth performance. Thr supplementation in the low CP diet might adversely affect the growth performance while Arg supplementation could have a beneficial effect on growth performance and oxidative status of birds during an acute infection stage.

Key Words: Broilers, *Eimeria*, Amino acids, Oxidative status

P292 Effects of methionine source and level on growth performance and intestinal health of *Eimeria*-infected chickens Guanchen Liu¹, Po-Yun Teng¹, Janghan Choi¹, Sudhir Yadav¹, Fengxian Wei², Woo Kim¹
¹University of Georgia, ²Institute of Animal Husbandry and Veterinary Science

The study was conducted to investigate the capability of L-methionine on reversing adverse effects of growth performance and intestinal health induced by methionine (met) deficiency and *Eimeria* infection in chickens. A total of 720 1-day old chicks (Cobb 500) were randomly allocated to 10 groups in a 2 x 5 factorial arrangement (6 replicates of 12 birds per cage) with diets and *Eimeria* challenge as the main factors. The treatment diets included 100%-DL-met, 100%-L-met (diets were formulated following to the standard Dig TSAA with DL-met or L-met supplementation, respectively), 80%-DL-met, 80%-L-met (diets contain approximately 80% Dig TSAA of the standard diets with DL-met or L-met supplementation, respectively), and the 60%-met (diet was formulated without met supplementation, approximately 60% Dig TSAA of the standard diets). At d14, challenge groups were gavaged with mixed *Eimeria* spp. Growth performance data in the starter phase were subjected to one-way ANOVA, whereas data from the challenge (d14-20) and recovery phases (d21-26) were analyzed by a 2-way ANOVA. In case of significant differences, orthogonal polynomial contrasts were used for post hoc comparison. The results showed that L-met groups (100% and 80% L-met) had significantly higher BWG and lower FCR than DL-met groups (100% and 80% DL-met) in the starter phase ($P<0.05$). However, the L-met groups did not show significant improvement on growth performance compared to the DL-met groups in the challenge and recovery phases, whereas 60%-met and *Eimeria* infection significantly reduced growth performance ($P<0.05$). The gut permeability was measured on 5- and 11-days post-infection (dpi). L-met groups had less gut permeability than the DL-met groups on 5 dpi ($P<0.05$). Furthermore, 100% met groups (100% DL and 100% L-met) reduced gut permeability compared to the other met groups (80 and 60%). In conclusion, L-met groups improved growth performance in the starter phase and gut permeability in the challenge phase.

Key Words: Methionine, Coccidiosis, *Eimeria*

P293 Influence of diet cereal grain and protein on broiler performance V. Reid¹UG, S. Wells-Crafton, C. Maynard, G. Mullenix, S. Orłowski, M. Kidd *Center of Excellence for Poultry Science, Division of Agriculture, University of Arkansas System*

To add to the body of literature pertaining to reduced crude protein (CP) diets in broilers, an experiment was conducted to evaluate the influence of diet cereal grain and CP on broiler performance for a 15 to 35 d grower period in Cobb MV x 500 male broilers. A factorial arrangement of six dietary treatments were fed, containing either corn or a corn-sorghum blend (50% ground and 50% whole sorghum) and formulated to 20, 18.75, and 17.5% CP. All diets were iso-caloric and adequate in all essential amino acids. Experimental diets were fed to eight replicate pens of twelve birds (0.08 m² per bird; 576 birds total), and live performance measurements were assessed for the 15 to 35 d period. On d 36, four randomly selected birds per pen were processed for evaluation of carcass traits and incidence of woody breast. All data were analyzed by two-way ANOVA using the GLM procedure of SAS 9.4, and means were separated using the F-protected Fisher's LSD test when appropriate ($P\leq 0.05$). Cereal grain x CP interactions were limited to fat pad yield, where broilers fed the 18.75% CP corn-sorghum blend diet had the lowest ($P<0.05$), 17.5% CP corn diet had the highest ($P<0.05$), and broilers fed all other combinations had intermediate fat yield ($P>0.05$). BW gain was reduced ($P<0.05$) for broilers fed the 17.5% CP diet compared with those fed the 20% CP diet, with broilers fed the 18.75% being intermediate ($P>0.05$). A stepwise reduction ($P<0.001$) in feed conversion was observed as CP was reduced. For carcass traits, broilers fed the 18.75 and 20% CP diets displayed increased carcass ($P=0.002$) and breast yields ($P=0.002$), and reduced fat pad yields ($P=0.014$), compared with broilers fed the 17.5% CP diets. No main effects ($P>0.05$) of cereal grain were observed for any measurement. Overall, varying dietary cereal grains did not improve live performance and carcass traits of broilers fed reduced CP diets.

Key Words: Amino acids, Broiler, Corn, Sorghum, Starch

P294 Determination of the Cobb 500 female broiler isoleucine requirement for a 22 to 42 d finisher period and evaluation of branched-chain amino acid synergism C. Maynard¹, G. Mullenix¹, S. Wells-Crafton¹, S. Rao², J. Lee³, M. Kidd¹ *1Center of Excellence for Poultry Science, Division of Agriculture, University of Arkansas System, 2Cobb-Vantress, Inc., 3CJ America - Bio Business*

An experiment was conducted to determine the isoleucine requirements for a 22 to 42 d finisher period in Cobb MV x 500 female broilers. A test diet was formulated to contain dietary isoleucine, valine, and leucine to lysine ratios of 56, 78, and 135, respectively. The test diet was then divided into seven lots and graded amounts of L-isoleucine were added to achieve isoleucine to lysine ratios of 56, 59, 62, 65, 68, 71, and 74. An additional control diet was formulated with isoleucine, valine, and leucine to lysine ratios of 65, 72, and 135, respectively. L-isoleucine, L-valine, and L-leucine were added to the control diet to construct additional controls: conventional isoleucine (65), low valine (72), and low leucine (135); conventional isoleucine, conventional valine (78), and high leucine (175); high isoleucine (71), conventional valine, and high leucine; conventional isoleucine, high valine (82), and high leucine; and high isoleucine, high valine, and high leucine. Digestible lysine was maintained at 1.10% in all experimental diets. The resulting diets were fed to 1,152 female broilers, placed in eight replicate pens of twelve birds, from d 22 to 42. Body weight gain, feed intake, and feed conversion were determined for the experimental period. At d 43, four birds from each pen were randomly selected and processed for evaluation of carcass traits and incidence of woody breast. Titration data were analyzed for linear and quadratic effects using PROC GLM of SAS 9.4 and all external controls were compared with the 65 isoleucine to lysine titration diet. Linear responses ($P<0.05$) were observed for feed conversion and breast (*Pectoralis major*) yield. No other significant responses ($P>0.05$) were observed for the isoleucine titration. Comparison among the controls generated a significant decrease

in fat yield when all three branched-chain amino acids were increased compared to birds fed the 65 isoleucine to lysine diet. The linear response observed for both feed conversion and breast meat yield indicate that optimal isoleucine levels may be beyond the 74 isoleucine to lysine ratio tested in this study.

Key Words: Isoleucine, leucine, valine, broiler

P295 Dietary crude protein and *Spirulina platensis* interaction on male broiler growth performance, water consumption and processing yields G. Mullenix¹, C. Maynard¹, S. Wells-Crafton¹, C. Maynard¹, S. Orłowski¹, W. Bottje¹, R. Brister², M. Kidd¹ ¹*Center of Excellence for Poultry Science, Division of Agriculture, University of Arkansas System, Tyson Foods, Inc.*

The anticipated increase in protein demand for animal feed dictates that multiple feed formulation strategies will need to be implemented in the future. Reducing dietary crude protein and the use of alternative protein sources, such as *Spirulina platensis*, are two possible solutions to alleviate the demand put on soybean meal. The objective of this study was to examine how male Cobb 500 broilers responded to reduced crude protein diets with and without a 2.5% dietary *Spirulina* inclusion. Six hundred and twenty-four, one-day old male chicks (Cobb500) were fed a common starter diet to 14 days of age then randomly assigned to an experimental treatment until 35d. Four experimental dietary treatments consisted of a 2 x 2 factorial design with *Spirulina* inclusion (0 and 2.5%) and CP level (17.75 and 20%) as the primary factors. Growth performance, feed intake, water intake, processing parameters, woody breast, breast colorimetrics, and footpad dermatitis were analyzed as randomized complete block design using a two-way ANOVA. Pen represented the experimental unit, with 10 replicates each. Data analysis were analyzed using JMP Pro15 statistical analysis software (SAS Institute, 2018) and statistical significance was considered at $P < 0.05$. Reducing dietary CP resulted in decreased BW gain (112g) and increased feed conversion (6 points). All yields ($p < 0.05$) reflected the decreased performance parameters observed when CP was reduced, as well as an increase in breast fillet redness (~14%), but lower average incidences of pododermatitis (~38%). Water intake per bird was decreased by lowering CP (430 mL) and from dietary inclusion of *Spirulina* (210 mL). However, *Spirulina* inclusion had mixed results as feed conversion (5 points) and feed intake (100g) were increased by its inclusion, yet no differences on BW gain were observed. Carcass and breast yields were negatively impacted by *Spirulina* inclusion, while breast fillet lightness (1%), redness (20%), and yellowness (27%) increased. Although both low dietary CP and dietary *Spirulina* reduced water intake, and understanding of poorer broiler performance from *Spirulina* inclusion and reduced CP in the test diets is warranted.

Key Words: Algae, Crude protein, Amino acid, Broiler

P296 Effect of methionine supplementation on growth performance, carcass traits, cell blood count, and intestinal morphology in broiler chickens at the grower period Zohreh Mehdipour^{*1}, Abolghasem Golian², Hassan Nassiri-Moghadam², Ali Javadmanesh², Chongxiao (Sean) Chen¹ ¹*North Carolina State University, 2Ferdowsi University of Mashhad*

Methionine (Met) is the first limiting amino acid in broilers. Meeting the requirements of Met is

crucial. This study was performed to investigate the effects of dietary Met levels on performance, carcass traits, blood cell counts and intestinal morphology in broiler chickens. 330 11-day-old male broilers (Cobb 500) were randomly divided into five dietary treatments (6 reps x 11 birds) They are: 85%, 100% (control), 115%, 130%, and 145% of Met requirement according to the Cobb 500 rearing guidelines. Birds and feeds were weighed at 11d and 22 d of bird age. Mortality was checked daily. At 23d of age, two birds per replicate were euthanized. Carcass traits (carcass yield, breast muscle, and thigh muscle) were measured. Duodenum was collected for intestine morphology analysis. Blood smears were made for blood cell

counts. Data were analyzed using GLM procedure. Differences among the means were compared using the Tukey's test. Linear and quadratic effects of dietary Met levels were investigated using polynomial contrasts (SAS 9.3, $P < 0.05$). Results showed that birds fed on the diets containing 100% and 115% level of Met supplementation had greater body weight gain (BWG) than 85% treatment ($P = 0.0002$). Furthermore, the birds fed with 115% Met improved feed conversion ratio (FCR) compared to the birds fed with 85% Met ($P = 0.006$). Increasing Met levels improved BWG ($P < 0.0001$) and FCR ($P < 0.0025$) in a quadratic trend. Carcass yield ($P = 0.006$) and breast muscle yield ($P = 0.001$) were increased in the groups fed with 115% Met compared with 85% and 145% of Met. And carcass yield and breast muscle yield increased quadratically ($P < 0.05$, $R^2 = ?$) with the increase of Met level. No statistical difference was observed in histological analysis and blood cell counts between treatments ($P > 0.05$). In summary, provide 115% of Met recommendation showed the most optimal growth performance and carcass traits at the grower period.

Key Words: Methionine, Broiler chicken, Growth Performance, Carcass Traits

P297 Impact of the timing and duration of increased dietary arginine on growth performance and processing characteristics of coccidiosis vaccinated broilers Trevor Lee¹, Jason Lee², Roshan Adhikari², Casey Owens¹, Samuel Rochell¹ ¹*Center of Excellence for Poultry Science, University of Arkansas System Division of Agriculture, 2CJ America, INC*

This experiment evaluated growth performance and processing characteristics, including pectorals major myopathies, of broilers vaccinated for coccidiosis and fed increased digestible Arg:Lys ratio levels during early or late feeding phases. A total of 2,002 Ross 708 male chicks were allocated to 91 floor pens (22 birds/pen; 13 replicate pens/treatment) and provided 1 of 3 corn-soybean meal diets that had: 1) an Arg:Lys ratio at 107 (107), 2) an Arg:Lys ratio at 115 (115), or 3) an Arg:Lys ratio at 125 (125). Dietary phases included a starter (0-12 d), grower (12-26 d), and finisher 1 (26-40 d) and 2 (40-53d) and met breeder nutrient recommendations. The elevated Arg diets were provided from either 0-26, 27-53, or 0-53 d to create 7 Arg:Lys ratio treatment combinations: 107'107 (0-26 d'27-53 d), 107'115, 115'107, 115'115, 107'125, 125'107, and 125'125. Feed intake (FI), BW gain (BWG), and FCR were evaluated, and fecal oocysts were counted at d 12. At d 53, 6 birds/pen were randomly selected for processing and p. major myopathy scoring. Data from 0-26 d were analyzed by a 1-way ANOVA and means were separated by Tukey's test ($P \leq 0.05$). The remaining data were analyzed by pre-planned orthogonal contrasts ($P \leq 0.05$). At d 12, oocyst shedding was similar ($P > 0.05$) among Arg levels. From 0-26 d, FI and BWG were not affected ($P > 0.05$) but FCR ($P = 0.007$) was higher for birds in the 115 group than those in the 107 and 125 groups. From 0-53 d, broiler BWG, FI, and FCR were similar ($P > 0.05$) among Arg levels regardless of feeding duration (early, late, or all phases). Wing and leg quarter weights, but not yields, were heavier ($P \leq 0.05$) for the 107'107 group compared with those fed increased Arg early or late. A lower severe incidence of woody breast occurred ($P = 0.013$) when increasing Arg levels early vs. the 107'107 group. A lower proportion ($P \leq 0.05$) of severe white striping was observed when increasing Arg levels (115 or 125) in early or late diet phases vs. the 107'107 group. In conclusion, increasing the Arg:Lys ratio above recommendations may reduce the incidence of severe muscle myopathies, but no effects on live performance or processing yields were observed in this trial when increasing Arg:Lys ratio during early, late, or all feeding phases.

Key Words: arginine, myopathy, vaccination, coccidiosis, broiler

Metabolism and Nutrition: Enzymes, Feed Additives

P298 Standardized ileal digestibility of amino acids in soybean meal with inclusions of exogenous carbohydrases for growing broiler chickens Tanner Wise^{*1GS}, Mike Blair², Olayiwola Adeola¹ ¹Purdue University, ²United Animal Health

Enspira (ESP) and Enspira⁺ (ESP⁺) are precision engineered blends of exogenous enzymes designed to degrade non-starch polysaccharides in common diet formulations. An experiment was conducted to determine the response of apparent and standardized ileal digestibility (SID) of amino acids (AA) in soybean meal to ESP or ESP⁺. Cobb-500 male broiler chickens were tagged, placed in battery cages, and fed a corn-soybean meal (SBM)-based starter diet. At 23 d of age, birds were individually weighed and 272 birds were allotted by body weight to 4 dietary treatments in a randomized complete block design. An N-free diet (10 birds/cage) and 3 corn starch-dextrose based semi-purified diets (8 birds/cage), formulated to contain 407.7 g/kg of SBM as the sole source of dietary N, were prepared, and 5 g/kg of TiO₂ was added as an indigestible marker. Enspira or ESP⁺ was added at 125 mg/kg to two SBM-containing diets, respectively, at the expense of dextrose. At 27 d of age, birds were euthanized, weighed, and digesta was collected from the terminal two-thirds of the ileum. Feed and digesta samples were dried and analyzed for dry matter, N, Ti, and AA content. The SID was determined by correcting the apparent ileal AA digestibility for the basal ileal endogenous AA losses determined using the N-free diet. Adding ESP or ESP⁺ increased ($P < 0.05$) the SID from 89 to 95% and 92 to 93% for Lys and Thr, respectively, as well as Arg, His, Ile, Leu, Ala, Ser, Asp, and Gly. None of the 2 enzyme blends had effect on the SID of Met, Val, Trp, Cys, or Tyr. Enspira⁺ reduced the SID of Phe ($P < 0.05$). These data indicate that including 125 mg/kg of ESP or ESP⁺ improves or has no detrimental effect on the SID of most of the indispensable and dispensable AA in SBM.

Key Words: amino acids, basal endogenous loss, broiler, standardized ileal digestibility

P299 Improved late lay hen performance and egg quality with the dietary supplementation of Q-Biotic® 1DP Claudia DeLeon^{*1GS}, Miloud Araba², Troy Lohrmann², Dawn Koltjes¹ ¹Iowa State University, ²Quality Technology International, Inc.

As hens age, egg production and quality decline. Direct-fed microbials (DFMs) are commonly used feed additives that could mitigate this decline in late lay hens. Therefore, the effects of the DFM, Q-Biotic® 1DP, a *Bacillus subtilis* strain, on egg production and egg quality of late-lay hens were investigated. Hy-line W-80 hens (n=288) were placed into 48 cages with 6 hens per cage at 60 weeks of age (woa). Cages were assigned to either 1) a control diet or 2) a diet containing Q-Biotic® 1DP (DFM). A baseline period (60-62 woa) preceded the 12-week experimental period (63-75 woa) during which hens were provided either the control or DFM diet. During both periods, daily egg production, egg weight (EW), and hen mortality were recorded. Weekly body weight (BW) and feed intake (FI) were collected with hen-day egg production (HDEP) and feed conversion ratio (FCR) calculated. To determine egg quality, eggs were analyzed for shell strength, Haugh unit, and yolk color thrice weekly using a DET6000. Data were analyzed for the main effects of dietary treatment, age, and the interaction between dietary treatment and age with the baseline period included as a covariate. Hen mortality rate, BW, and FI were similar between treatments ($P > 0.05$). Average HDEP was different across dietary treatments and age ($P < 0.04$), but not the interaction ($P > 0.05$). Inclusion of Q-Biotic® 1DP in the diet increased average HDEP compared to the control diet by 1.78% and HDEP declined over time. Additionally, average EW was different between dietary treatment and age ($P < 0.01$), but not the interaction ($P > 0.05$). Inclusion of Q-Biotic® 1DP in the diet increased EW compared to the control diet by 0.5g and EW increased throughout the trial. Despite the changes in EW and HDEP, FCR was similar between dietary treatments ($P = 0.70$). Q-Biotic® 1DP minimally influenced egg

quality. Shell strength and yolk color were elevated ($P < 0.03$) in eggs from hens on the control diet; however, egg quality measurements for both control and DFM treatments were within acceptable ranges. In conclusion, inclusion of Q-Biotic® 1DP increased egg production and EW but did not alter hen mortality, BW, FCR, or egg quality in late lay hens.

Key Words: laying hen, egg production, DFM, Q-Biotic

P300 In-vitro digestive assay to understand the effect of probiotics and a prebiotic and essential oil blend on Salmonella Enteritidis Ishab Poudel^{*1GS}, Alamanda Calvert², Pratima Adhikari¹ ¹Mississippi State University, ²BioMatrix International

This study evaluated the effect of probiotics, a prebiotic and essential oil blend and their combination on the growth of *Salmonella* Enteritidis (SE) in a digestive assay mimicking three digestive compartments of the poultry digestive tract: crop, proventriculus, and intestine. A poultry commensal *Lactobacillus reuteri* (LR) and SE strain were utilized. The prebiotic product was included at 0.025% (w/w) and probiotic product, a combination of 3 strains of *Bacillus* spp., at 2×10^6 cfu/g in the feed. This experiment was a completely randomized design with the treatments: Positive control (PC); PRE = PC + prebiotic; PRO = PC + probiotic; and PRE + PRO. In the crop, 5 g of peaking layer feed (4.5% Ca), 1 ml each of SE (2×10^6 cfu/ml) and LR (2×10^6 cfu/ml), and 0.03 M hydrochloric acid (HCL) were added. pH was adjusted to 5.2 with HCL as needed and tube mixed by shaking vigorously and incubated for 30 min at 40°C. The proventriculus was simulated by adding pepsin and 1.0 M HCL to reduce the pH to 3.0 and incubated for 45 min at 40°C. Intestinal conditions were simulated by adding pancreatin and sodium bicarbonate to adjust the pH to 6.4 to 6.8 and incubated for 2 h at 40°C. Total bacterial count changes were recorded for each compartment by spread plate technique and incubated at 37°C for 48 h. XLT4 and MRS were used for SE and LR and incubated aerobically and anaerobically, respectively. Heat shock was applied at 80°C for 10 mins before enumerating *Bacillus* on TSA agar aerobically. Data were log-transformed and subjected to ANOVA using the PROC GLM procedure of SAS (V9.4), means were separated using least-squares means. An interaction between compartment and product occurred for total counts of SE ($P < 0.001$). In the intestine, PRE, PRO, or PRE+PRO significantly reduced the total count of SE compared to the PC ($P < 0.05$). *Bacillus* and LR total counts increased from crop to proventriculus to intestine ($P < 0.001$). PRE and PRO products were able to reduce growth of SR *in-vitro* but did not show additive effect. The amount of HCL was more than what has been previously used in similar digestibility assays. Follow-up *in-vitro* experiments are needed to validate if the efficacy of these products differs when using broiler or layer diets due to dietary Ca buffering capacity.

Key Words: Prebiotic, Probiotic, Digestive assay, in-vitro, Salmonella

P301 Effect of a novel non-antibiotic feed additive on intestinal health and broiler performance with a coccidiosis vaccination program and natural necrotic enteritis occurrence Daniel De Leon^{*1GS}, Rosana Hirai¹, Austin Silva¹, Macey Randig¹, Garrett Powell¹, Omar Gutierrez², Staci Cantley², Audrey McElroy¹ ¹Texas A&M AgriLife Research, ²Huvepharma Inc.

This experiment was conducted to determine the impact of feeding a novel non-antibiotic additive, Clarity-Q (CQ), a granulated extract of *Quillaja saponaria*, on growth performance, intestinal morphology, intestinal lesion scoring, litter moisture (LM), and footpad dermatitis (FPD). Ross 308 male broilers were fed corn-soy diets formulated to meet or exceed breed nutrient requirements from D0-42 with a highly soluble source of calcium as a risk factor for naturally occurring necrotic enteritis. Fifteen replicate pens of 22 chicks each were spray-vaccinated for coccidia (Advent®) and randomly allocated to 1 of 5 treatments (TRTS): Negative control (NC); Bacitracin methylene disalicylate at 50g/ton (PC); CQ at 15g/ton (CQ15);

CQ at 30g/ton (CQ30); or Magni-Phi (MP) at 250g/ton. On D15, D30, and D42, BW, BW gain (BWG), feed intake, and mortality-corrected FCR were measured corresponding with feeding phase changes. On D15 and D30, intestinal samples were collected (1 bird/pen) for morphology and 2 birds/pen were evaluated for macroscopic intestinal lesions from *Eimeria* spp. and microscopic oocyst counts for *E. maxima*. On D15 and D42, 5 samples of litter/pen were collected to measure LM, and 5 birds/pen were evaluated for FPD lesions. Data was analyzed via SAS using the GLM procedure with a randomized complete block design and significance at $P < 0.05$. Tukey's range test was used for mean separation. From D0-15, CQ15 and CQ30 resulted in improved BW, BWG, and FCR compared to NC or MP, with similar performance to PC. Similarly, from D16-30, CQ TRTS had improved BW and BWG compared to NC. Overall, the most efficient FCR occurred in the CQ TRTS. CQ15 and CQ30 increased D15 LM when compared to NC, but no difference was seen at D42. CQ15 and CQ30 increased the incidence of FPD compared to all other TRTS at D15 and D42 compared to the NC. D15 villi height was increased with CQ15 when compared to NC. No differences were observed in mortality or macroscopic lesion scores. This data suggested that inclusion of CQ was beneficial for performance through D42, compared to the PC. Additional research should include metrics for FPD and examine the influence of the product on intestinal alterations contributing to increased LM observed here.

Key Words: Necrotic Enteritis, Coccidiosis, Clarity-Q, Growth Performance, Intestinal Health

P302 Effects of syngenta enogen corn on growth performance, body composition, egg production, egg quality, and skeletal development in layers 18-45 weeks Dima White^{GS}, Woo Kyun Kim, Milan Sharma, John Gonzalez *University of Georgia*

Previously our lab provided Syngenta Enogen® corn (Syn corn) to pullets with positive effects on growth, intestinal & skeletal development. In continuation, we believe it could be beneficial for layers. We hypothesized that an alternative corn, Syn corn, with higher amylase & energy levels for better digestion could be used in layers to improve maintenance & performance. A study was done to explore the effects of Syn corn on growth performance, body comp., egg production, egg quality & skeletal development in layers. Total of 320 18 wk-old Leghorn hens (80 hens/trt) randomly allocated to 4 trt groups & raised 45wks. Diet trts: 1)(PC):Conventional(Conv) poultry diet containing corn&soybean meal, 2)(NC):PC – 200 kcal/kg ME, 3)(PCS):PC replaced with Syn corn & 4)(NCS):NC replaced with Syn corn. Egg production daily. At 20, 25, 30, 35, 40&45wks, feed consumption, body weight, feed efficiency, egg production/quality measured. At 45 wk, body comp. (fat weight, lean weight, fat %, lean %, bone mineral content(BMC) & bone mineral density(BMD)), and bone 3D structural analyses using micro-Ct measured. Data subjected to one-way ANOVA using GLM procedure, means deemed significant at $P < 0.05$. Results indicated significant decrease ($P = 0.0071$) in feed intake for Syngenta diet(PCS) vs normal corn diet(PC&NC), while maintaining increased body weight gain(BWG) at 45wk. Significant decreases in FCR/dozen eggs at 35($P = 0.0036$) & 45wks($P = 0.0014$) for PCS vs PC&NC. Body comp., numerically(NUM), PCS&NCS had higher muscle %, muscle weight, total weight, & BMC vs PC&NC at 45wk. Egg production, significant increase in production at 35($P = 0.0036$), 40($P = 0.0012$) & 45wks($P = 0.0231$) for PCS vs PC&NC. Egg quality, significant increase in egg wt. for PCS at 45wk($P = 0.0078$) vs PC&NC. NUM, PCS&NCS had higher albumen wt., specific gravity, Haugh unit, yolk wt. & shell wt. vs PC&NC, but NCS had higher shell thickness, yolk % & shell % vs PC&NC. Micro-Ct, significant difference with PCS&NCS being highest($P = 0.0497$) in trabecular bone vs PC&NC for 45wk. NUM, PCS&NCS higher in bone parameters for tissue volume, BMD, BMC & trabecular bone structure vs PC&NC at 45wk. In conclusion, feeding

layers Syngenta corn can possibly improve growth performance, body comp., egg production/quality & bone health.

Key Words: syngenta corn, layer, egg quality, DEXA, bone 3D structure

P303 Evaluate the effects of Syngenta Enogen corn on growth performance, intestinal development, body composition and skeletal development in pullets from 0 – 17wks Dima White*, Woo Kyun Kim, Milan Sharma, John Gonzalez *University of Georgia*

Corn is a produced feed grain in U.S. accounting > than 95% of production. Corn crop provides main energy ingredient in livestock feed including poultry. Rising grain costs due to demands means more challenges to poultry sectors. An alternative corn, Syngenta Enogen® corn (Syn corn), with higher energy & amylase activity for better digestion could be used in poultry to offset demand for conventional(Conv) corn. We hypothesized Syn corn could improve pullet maintenance parameters. A study was done to explore effects of Syn corn on growth performance, intestinal development, body composition & skeletal development. Total of 600 one-day old Leghorn pullets (4trt x 10rep x 15 birds/cage) randomly allocated to 4trt groups & raised to 17wk. Diet trts: 1)(PC):Conv poultry diet with Conv corn, 2)(NC):PC – 200 kcal/kg ME, 3)(PCS):PC replaced with Syn corn & 4)(NCS):NC replaced with Syn corn. At 6, 12 & 17wks, feed consumption, body weight & feed efficiency recorded. Bird from each cage euthanized to measure body composition(fat weight, lean weight, fat %, lean %, bone mineral content(BMC) & bone mineral content(BMD) using Dual energy x-ray absorptiometry(DEXA). Intestinal samples for morphology(villi height, crypt depth & ratio). At 17wk, femur bone samples for 3D structural analyses using Micro-CT(CT). Data subjected to one-way ANOVA using GLM procedure with means deemed significant at $P < 0.05$. Results indicated significant decrease ($P = 0.0492$) in feed intake for Syngenta diet(PCS) compared to normal corn diet(PC) while maintaining a higher body weight(BW) & increased body weight gain(BWG) for starter period. Numerically(NUM) PCS&NCS had lower FCR & higher BW&BWG than PC&NC for developer period & 17wk period. Intestinal development results, NUM, PCS&NCS had higher ileum, jejunum & duodenum villi height compared to PC&NC for 17wk period. DEXA results, NUM, NCS was higher in muscle wt., % & body weight compared to PC&NC for 17wk period while PCS was higher in BMD compared to PC&NC. CT results, PCS&NCS were NUM higher in bone parameters for tissue volume, bone volume, BMD, BMC, cortical parameters & trabecular parameters compared to PC&NC for 17wk. In conclusion, feeding pullets Syn corn vs Conv corn can possibly improve growth performance, intestinal health & bone health.

Key Words: syngenta Corn, pullet, amylase, DEXA, bone 3D structure

P305 Effect of multi-strains probiotic supplementation on broilers' growth and prevention of Salmonella infection

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Two trials were conducted in determining a newly developed multi-strains probiotic as an alternative to AGP in broilers. In the first trial, 280 DOC ROSS 308 broilers were randomly allotted into 4 TRTs including control group (CT), AGP group (Enramycin 10 ppm, AB), commercial probiotic (CM, 10⁶ CFU/g), and SYNLAC multi-strains probiotic group (SYN, 10⁶ CFU/g), with 5 replications per treatment and 14 birds per pen. All feeds were pelleted and fed to birds for 35 d. Birds fed AB and SYN diets had increased weight gain (p<0.05) and FCR (p<0.05) as compared to the CT from d-0 to d-21; however, no significant differences were observed between groups from d-21 to d-35. And the European Production Index was 362.7 (CT), 423.5 (AB), 368.1 (CM), and 386.9 (SYN), respectively (p<0.05). In the second trial, *Salmonella* challenge in broiler was investigated. 72 DOC ROSS 308 were randomly distributed into control with no challenge (CT), challenged control group (PC), challenged with SYN-LAC probiotic mixed in feed (SYN_F), and SYNLAC probiotic mixed in water and spray (SYN_S), each TRT had 3 replicates with 6 birds per rep. SYN_S chicks received probiotics water spray (10⁷ CFU/bird) on d-1 only, and SYN_F chicks fed dietary probiotics (10⁶ CFU/g) during the entire experimental period. At d-5, chicks of PC, SYN_F, and SYN_S groups were infected with *Salmonella typhimurium* (10⁷ CFU/bird) and clinical traits were observed after 3 and 6 days of challenge. *Salmonella* invasion in the spleen and liver were increased in PC group as compared to CT group (p<0.05), however, birds treated with SYN_F or SYN_S had less spleen and liver invasions than the PC. As compared the small intestinal morphology, SYN_F and SYN_S had lower crypt depth and higher villus height than PC after 6 days challenge (p<0.05). In summary, the newly developed multi-strains probiotic showed an improvement of broiler growth and reduction of *Salmonella* infection, it suggests that SYNLAC probiotic may be used as an alternative to AGPs in broilers.

Key Words: probiotic, broiler, growth performance, *Salmonella*, intestinal morphology

P308 The efficacy of essential oils in improving egg production, egg quality, and gut health of laying hens Basheer Nusairat^{*1}, Grayson Walker², kamel Mahmoud¹, Mohammad Diya¹ Obeidat¹ ¹*Jordan University of Science and Technology*, ²*North Carolina State University*

A trial was conducted to evaluate the efficacy of essential oil supplementation on laying hen egg production and quality, and gut health. Fifty-four Lohman LSL-Classic laying hens were randomly assigned to 3 treatments and provided different levels of a liquid essential oil blend containing Citrus, Oregano, and Anise oils in drinking water from 31 to 39 weeks of age. Hens received a control treatment (no essential oils), essential oils level-1 (30 ml per 1000 ml of water), or essential oils level-2 (60 ml per 1000 ml of water). The layer diet was formulated according to the specifications listed in the nutrition guidelines of Lohman LSL-Classic and fed throughout the experimental period. Each of the 3 treatments had 6 replicates of 3 hens each. Data were analyzed as a one-way ANOVA using the GLM procedure. No significant differences were observed in egg production data between the groups. Heavier egg weights ($P<0.01$) were observed in the control treatment followed by level 2 and then level 1 for weeks 1 and 3. However, egg weight was heavier ($P<0.0001$) for levels 2 and 1 compared to the control treatment at week 4. No significant difference was observed for shell thickness. Haugh units were higher ($P<0.01$) for level 2 compared to control and level 1 at week 4. At the end of the study, jejunum villi length and crypt depth were higher ($P<0.01$) for levels 2 and 1 compared to the control treatment. There were no significant differences among ileum measurements or blood chemistry parameters at any point during the experiment. Based on these results, it was concluded that adding essential oils to drinking water positively improved jejunum histology and resulted in heavier egg weights in Lohman laying hens.

Key Words: Layers, essential oils, egg production, histology

P309 Effect of single and multi-strain Q-Biotic® Bacillus on performance of broiler chickens Miguel Ruano¹, Troy Lohrmann¹, Miloud Araba^{*1}, Brett Lumpkins², Greg Mathis² ¹*Quality Technology International, Inc.*, ²*Southern Poultry Research, Inc.*

The ban or reduction of AGP use in poultry production has increased the adoption of direct fed microbials (DFM). Two experiments were conducted using built up litter to evaluate the effect of a single-*Bacillus* strain (DFM1; Q-Biotic® 1DP) and a 3-*Bacillus* strain (DFM2; Q-Biotic® 3DP) on growth performance of broilers. In experiment 1, 1250 d old male Cobb 500 broilers were randomly assigned to 5 dietary treatments with 10 floor pens per treatment and 25 birds/pen and fed to 45 d of age. In experiment 2, 1100 d old male Cobb 500 broilers were randomly assigned to 5 dietary treatments with 10 floor pens per treatment and 22 birds/pen and fed to 42 d of age. Treatments consisted of a negative control (NC), positive control (PC; BMD@50, 50g/ton), DFM1 (500K cfu/g feed), DFM2 (700K cfu/g feed), and commercial probiotic (DFM3; 500K cfu/g feed). BW and FI were recorded at start of experiment, 28 d and 42 d of age, and mortality daily. In experiment 1, five birds per pen were randomly

selected for processing at 45 d of age. Data were analyzed by ANOVA, and statistical significance considered at $P\leq 0.05$. In experiment 1, feeding PC or DFM2 improved ($P<0.05$) BWG by 255 and 243 grams, and reduced ($P<0.05$) mortality-corrected FCR by 12 and 17 points at 42 d of age, respectively, compared to NC. Birds fed DFM3 or DFM1 had lower BWG than PC and DFM2, but were not different from NC. DFM1 and DFM3 reduced ($P<0.05$) mortality-corrected FCR by 10 and 12 points, respectively, compared to NC, but did not significantly differ from PC and DFM2. Compared to NC ($P<0.05$), higher % breast, lower carcass weight, and lower % carcass were observed with DFM2, DFM3, and DFM1, respectively. In experiment 2, compared to NC at 42 d of age, PC increased ($P<0.05$) BWG by 124 grams, which was greater ($P<0.05$) than DFM3 but was not statistically different from DFM2 or DFM1. PC and DFM2 improved ($P<0.05$) mortality-corrected FCR by 12 and 11 points compared to NC, whereas DFM1 and DFM3 improved ($P<0.05$) FCR by 9 and 4 points, respectively. Mortality-corrected FCR of PC and DFM2 were lower ($P<0.05$) than that of DFM1, which was lower ($P<0.05$) than DFM3. In conclusion, all treatments improved broiler performance compared to NC, with best performances observed with the 3-strain Q-Biotic 3DP or BMD50.

Key Words: Broiler chickens, Performance, BMD, DFM, Q-Biotic

P210 Effects of a novel ZEN-degrading enzyme (ZENzyme®) on the performance of laying hens fed zearalenone-contaminated diets Verena Starkl^{*1}, Bettina Behler-Woechtl¹, Ursula Hofstetter¹, Barbara Doupovec², Markus Aleschko² ¹*Biomim Holding GmbH*, ²*Biomim Research Center*

Zearalenone (ZEN) is amongst the three most prevalent mycotoxins worldwide. Corn substantially contributes to the overall contamination of feed, as it is the most affected commodity in the USA (2015-10/2021: 36% prevalence, 287 ppb average contamination). The toxin is responsible for a wide range of estrogenic and toxic effects in different species. The best known - but not exclusive - effects in livestock are on reproductivity. In layers, ZEN causes histological changes of the reproductive tract, egg shell alterations and cysts in the oviduct.

So far, there was no satisfying solution available to specifically counteract ZEN in feed. With ZENzyme, BIOMIN developed an enzymatic solution to detoxify ZEN. The recombinant enzyme specifically hydrolyzes ZEN to the non-toxic/non-estrogenic metabolite HZEN.

The aim of the experiment was to evaluate the efficacy of the hydrolase in laying hens.

For the trial, 96 Lohmann Brown laying hens were allocated to three experimental groups: a negative control, a positive control exposed to 400 µg/kg ZEN and a treatment group (400 µg/kg ZEN + 20 U hydrolase/kg feed). The trial started with hens aged 31 weeks and lasted for 14 days. Laying rate (%), average egg mass (g) and FCR (g feed/g egg mass) were assessed. Feces was sampled on day 14 to measure ZEN and its metabolite HZEN as biomarkers.

For statistical analysis (IBM SPSS Statistics 22), a test for normal distribution was performed, depending on the result, either the non-parametric Mann-Whitney U-test or a t-test were used for the biomarker data.

Biomarker analysis proved significant reduction of ZEN in excreta of the treatment group compared to the ZEN group (203 and 294 ng/g, respectively), whereas HZEN was only detected in excreta of the treatment group (67 ng/g). Treated animals tendentially performed better than ZEN exposed but not treated animals. Laying rate was 94.79% in treatment group and 90.78% in ZEN group, respectively. Treatment resulted in a higher average egg mass (61.67 g) and a lower FCR (1.73) compared to the positive control (60.60 g average egg mass, 1.80 FCR).

The results of the trial confirm the effective enzymatic biotransformation of ZEN to non-toxic/non-estrogenic HZEN in laying hens. The treatment resulted in an overall improvement of performance.

Key Words: zearalenone, ZENzyme, layer, enzyme, mycotoxin

P311 Effect of supplementation of a mycotoxin mitigation product on physiological and immune changes in broilers fed mycotoxin contaminated diets Sangita Jalukar¹, Yong Fang², Yutong Fu², Lihong Zhao², Jinglin Ma², Feifei Han² ¹*Arm and Hammer Animal and Food Production*, ²*State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University*

A study was conducted to test the physiological and immunosuppression effects of supplementation of a mycotoxin mitigation product in broilers fed diets contaminated with mycotoxins. One-day-old Arbor Acre male broilers received one of six different treatments made up of 2 diets formulated with clean or moldy corn (AFB1 130 ppb, DON=1.5 ppm, ZEN=496 ppb), and 3 inclusion rates of test product (BG-MAXTM, Arm and HammerTM) 0 kg/MT, 1 kg/MT and 2 kg/MT arranged in a 2 x 3 factorial design. The trial was conducted in 8 replicate cages per treatment with 10 birds per cage for 42 days. Serum and jejunal tissue samples were collected on d42 to measure oxidative stress, gut morphology and integrity, and some indicators of immune status of the birds. The data were analyzed statistically with significance noted at $P < 0.05$. Broilers fed mycotoxin contaminated diets had higher expression of cellular oxidative stress markers HMOX and XOR compared to broilers fed all other treatment diets. Expression of gut integrity markers CLDN1, CLDN2 and ZO1 was reduced in broilers fed mycotoxin contaminated diet. Broilers fed diets supplemented with BG-MAX had similar CLDN2 expression as broilers fed the control diet, while expression of CLDN1 and ZO1 was intermediate. BG-MAX supplementation in the control diet had a positive affect and mycotoxin contamination had a negative affect on villus height (VH) and VH/crypt depth (CD). Broilers fed diets supplemented with BG-MAX at the higher inclusion rate in the mycotoxin contaminated diet, had increased VH and VH/CD similar to control fed broilers. Serum level of total antioxidant capacity (T-AOC) and Glutathione Peroxidase (GSH-PX) were reduced and that of MDA and 8-OHdG was increased in broilers fed mycotoxin contaminated diets compared to those fed control diet or mycotoxin contaminated diet but supplemented with BG-MAX. Expression of Toll-like receptor 4 was increased and antibody titers to routine vaccinations were reduced in broilers fed mycotoxin contaminated diet compared to control and broilers fed the mycotoxin contaminated diet supplemented with BG-MAX. In conclusion, supplementation of diets with BG-Max reduced negative physiological and immunosuppression effects of mycotoxins contaminated diets in broilers.

Key Words: mycotoxins, broilers, gut integrity, immunosuppression, oxidative stress

P312 Influence of an enzyme blend (Immuzyme) on broiler performance from 0-42 days Matthew Jones^{*1}, Charles Hofacre¹, Yulin Ma², G. Zhang³, L. Deng³, L. Zhang³ ¹*Southern Poultry Research Group*, ²*Hanley International, LLC*, ³*Tianjin Biofeed Technology Co., LTD*

Supplemental enzymes in poultry diets release nutrients which would typically be unavailable to the birds. Those that degrade non-starch polysaccharides (NSPs) may also alleviate intestinal stress by reducing this antinutritional factor in the intestinal lumen. Immuzyme is an enzyme blend that targets these polysaccharides. In a floor pen model, nine replicate pens of 25 male Ross chicks were placed on fresh pine shavings in each of five dietary treatments. Treatments included a positive control (PC) diet formulated to primary breeder recommendations, PC + Immuzyme (0.02%), a negative control (NC, PC-120 Kcal/kg) lower energy diet, as well as this diet supplemented with Econase XT (0.01%) or Immuzyme (0.02%). Birds and feed were weighed on day 0, 14, 28, and 42 for performance evaluation. Data were subjected to an Analysis of Variation and compared using Least Significant Difference. P -value ≤ 0.05 was considered significant. From 0-14 days, there were no differences in body weight gain

or feed conversion adjusted for mortality (aFCR). Body weight gain was not different from day 0-28. PC (1.42) and PC + Immuzyme (1.41) had significantly lower aFCR compared to NC (1.46) and NC + Econase XT (1.48) at 28 days. NC + Immuzyme was intermediate between the two groups (1.45). From 0-42 days, the aFCR was higher in the NC + Econase XT group compared to the other treatments. Weight gain was greater in the NC group compared to the PC + Immuzyme and NC + Econase XT group. Mortality was significantly higher in the NC group compared to the PC and NC + Econase XT groups. In challenge experiments, it is a routine observation that groups with greater mortality exhibit better compensatory gain and performance in the post challenge interval. Adjusting for mortality, feed intake was numerically highest in the NC group (96.51kg/pen). There was no difference in performance between the PC and the NC + Immuzyme from 0-42 days. In this study with no intestinal challenge, Immuzyme had potential energy sparing influence and helped the birds manage dietary and environmental stressors. Future studies with added intestinal stress, such as a high NSP diet or a NE challenge, may demonstrate the product's efficacy in mitigating these challenging situations.

Key Words: Energy, NSPase, Xylanase, β -Mannanase, intestinal health

P313 Effect of an essential oil blend on growth performance of broilers under different coccidiosis control programs 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, especially for AGP free production. However, their effect is inconsistent, which is probably associated with different gut health challenges encountered. A floor pen study with 1584 Ross 308 male broiler was conducted to evaluate the effect of an essential oil blend (EOB, NEXT ENHANCE[®] 150, 1:1 thymol carvacrol) on growth performance of young broilers as affected by coccidiosis control program. The study consisted of 3 coccidiosis programs, none, vaccination (1X on d 0 by oral gavage), and ionophore (monensin 90g/ton). Within each program, diets were either supplemented with 0 or 60 g/ton EOB, resulting in 6 treatments. Each treatment had 12 replicate pens of 22 birds/pen. Nutritionally complete typical US corn soybean meal based diets containing DDGS and MBM were fed throughout the trial. Body weight, feed intake, feed conversion ratio (FCR), and mortality were determined on d 14, 28, and 34. Performance index was calculated as average daily gain x livability / FCR x 100. Data were subject to one way ANOVA and means were separated by Fisher's protected LSD test. On d 14, in the absence of EOB, vaccination increased FCR and reduced performance index ($P < 0.05$), reflecting cost for immunity development, and ionophore increased body weight and improved FCR ($P < 0.05$). In the presence of EOB, no negative effect of vaccination was observed ($P > 0.05$) and ionophore increased body weight, improved FCR and performance index on d 14 ($P < 0.05$). Essential oil blend increased performance index of the vaccinated broilers on d 14 ($P < 0.05$). On d 28 and 34, ionophore fed birds had better body weight, FCR, feed intake and performance index ($P < 0.05$) and no difference was seen between control and vaccinated birds ($P > 0.05$). On d 34, the birds without any cocci control program had the worst FCR, EOB improved FCR of these birds ($P < 0.05$). Therefore, the beneficial effect of EOB was dependent on coccidiosis control programs and was associated with coccidia challenge, suggesting EOB was able to alleviate some of the negative impact of coccidiosis on growth performance of broilers

Key Words: Essential oil blend, coccidiosis vaccine, broiler, coccidiosis

P314 Effects of dietary supplementation of medium chain fatty acids on gut health in commercial male broiler chickens Cinta Sol^{*}, Mónica Puyalto, Juan José Mallo *NOREL S.A.*

With the current bans and limitations on AGP all around the world, feed additives are widely used to inhibit the effects of pathogenic microorgan-

isms on poultry health. Medium-chain fatty acids (MCFAs) have become a focus of attention due to their potentially favorable antimicrobial effects and performance booster. This study was designed to evaluate two feeding strategies that can replace in-feed antibiotics by MCFA from vegetal origin.

A total of 400 male Cobb 430 broiler chickens were randomly distributed into four treatments (n=10): NC, negative control, without any in-feed antibiotic growth promoter; PC, positive control, NC diet supplemented with bacitracin methylene disalicylate (BMD, 50 ppm); DIC1, NC diet supplemented with sodium salt of MCFA (Dicosan®, NOREL SA) at 1.5 g, 1.0 g and 0.5 g per kg diet during the starter, grower and finisher stages; and DIC2, NC diet supplemented with sodium salt of MCFA (Dicosan®, NOREL SA) at 1.0 g, 0.5 g and 0.5 g per kg diet during the starter, grower and finisher stages. Chemical coccidiostat (Diclazuril, 0.3g/kg) was used throughout the study period.

Performance, carcass traits, microbial population and histomorphology in small intestine were evaluated at the end of the trial (Day35). All parameters were analyzed using a one-way ANOVA via SAS, with pen as the experimental unit and values were compared by Tukey's test.

At the end of the study (35d) DIC1 birds were numerically the heaviest (2410g, 2421g, 2453g and 2419g, for NC, PC DIC1 and DIC2, respectively, $P>0.05$) without differences in FCR or in carcass traits. Those slight differences could be related with a significant shift in the microbial populations (log10 CFU/g digesta) of *E. coli* (7.17c, 4.66ab, 4.49a and 4.78b for NC, PC DIC1 and DIC2, respectively, $P=0.0001$); *Salmonella spp.* (6.82c, 4.66b, 4.29a and 4.90b for NC, PC DIC1 and DIC2, respectively, $P=0.0001$); *Cl. Perfringens* (6.32d, 5.59c, 5.27b and 4.84a for NC, PC DIC1 and DIC2, respectively, $P=0.0001$). There were no significant differences in small intestinal morphology, however, DIC2 had numerically higher villus length and Crypt depth than the other treatments.

In conclusion, this study supports the idea that DICOSAN can improve gut health and can replace BMD as an antibiotic growth promoter.

Key Words: medium-chain fatty acids, gut health, broilers

P315 Effects of dietary supplementation of medium chain fatty acids as a performance booster in commercial male broiler chickens Cinta Sol*, Mónica Puyalto, Juan José Mallo NOREL, SA

This study was designed to evaluate two feeding strategies that can replace the inclusion of in-feed antibiotics by medium-chain fatty acids (MCFA) from vegetal origin.

A total of 400 male Cobb 430 broiler chickens were randomly distributed into four treatments (n=10): NC, negative control, corn-soybean meal diet without any in-feed antibiotic growth promoter; PC, positive control, NC diet supplemented with bacitracin methylene disalicylate (BMD, 50 ppm); DIC1, NC diet supplemented with sodium salt of MCFA (Dicosan®, NOREL SA) at the rate of 1.5 g, 1.0 g and 0.5 g per kg diet during the starter, grower and finisher stages respectively; and DIC2, NC diet supplemented with sodium salt of MCFA (Dicosan®, NOREL SA) at the rate of 1.0 g, 0.5 g and 0.5 g per kg diet during the starter, grower and finisher stages respectively. Chemical coccidiostat (Diclazuril, 0.3g/kg) was used throughout the study period.

The trial lasted 35d, and performance parameters were recorded weekly. Data were analyzed using a one-way ANOVA via SAS, with pen as the experimental unit and values were compared by Tukey's test.

Regarding body weight, there were significant differences at d7 being the birds from NC and DIC1 heavier than DIC2 (217g^a, 212.8g^{ab}, 215.1g^b and 207.9g^a for NC, PC DIC1 and DIC2 respectively, $P=0.007$). At 14d, a tendency was observed where NC, PC and DIC1 tended to be different from DIC2 (558.7g, 555.8g, 552.1g and 537.2g for NC, PC DIC1 and DIC2, respectively, $P=0.06$). No more significant differences were observed in body weight along the study period, however, at the end of the study (35d)

DIC1 birds were the heaviest (2410g, 2421g, 2453g and 2419g, for NC, PC DIC1 and DIC2, respectively). There were not significant differences in the FCR among treatments, numerically DIC1 had the lowest FCR globally (1.52, 1.49, 1.46, 1.52 for NC, PC DIC1 and DIC2, respectively). There was no statistically significant effect of the dietary treatments on mortality, productivity index (EPEF) and carcass traits of the birds.

In conclusion, this study supports the idea that DICOSAN can replace BMD as an antibiotic growth promoter. The effects observed in this trial could be related with an improvement of gut health status.

Key Words: medium-chain fatty acids, performance, broilers

P317 Effect of a mix of essential oils and short chain fatty acid supplementation on growth performances and litter quality of broilers Quentin Pagé, Claire Le Dain, Simon Bourbouze* MiXscience

The aim of this study was to evaluate two nutritional strategies to enhance performances : a mix of thymol, carvacrol and butyric acid (Lumigard TCB) and an antibiotic growth promoter (AGP). Lumigard TCB was conceived with slow release technology (VStar). The trial was conducted in the university of Sao Paulo (Brazil). A total of 432 days-old Cobb 500 male chicks were randomly allocated to three dietary treatments, each replicated 12 times. Software R[®] was used for statistical analysis. Dietary treatments were : Negative control (NC, no additive), NC with MiX-science product (LTCB) and NC with Enramycin as AGP (AGP). Performance was measured during the two phases of the trial (starter 0-21d and finisher 22-35d). Litter was sampled in each floor pens at different ages (7,

14, 21 and 28 days) to analysis dry matter content. At 21 days, we have observed little differences between groups for both the body weight (BW) and the average daily gain (ADG). A significant decreased of average feed intake (AFI; -2.08 g/d; $p < 0.05$) was observed for LTCB group compared to the NC group leading to a significant improvement in feed conversion ratio (FCR; -0.08 g/g; $p < 0.05$). No differences for AFI and FCR between AGP group and NC group were observed. At 35 days, no differences were shown between the three treatments for BW and ADG, while LTCB group significantly decreased AFI (-2.6 g/d compared to NC, -5.3 g/d compared to AGP; $p < 0.05$) and improved FCR (-0.064 g/g compared to NC, -0.075 g/g compared to AGP; $p < 0.05$). At 7 days, dry matter content was significantly higher both for AGP (+5 %; $p < 0.05$) and LTCB (+5.2 %; $p < 0.05$) groups. The litter dry matter content for LTCB group was the highest at 21 day (-1.1 % compared to NC, -0.9 % g/d compared to AGP; $p < 0.05$). This trial suggests that the feed supplementation with Lumigard TCB improved feed efficiency and the quality of litter. Therefore Lumigard TCB could be part of the global strategy to reduce AGP supplementation.

Key Words: essential oils, fatty acid, growth performances, litter quality

P318 The effect of dietary supplementation of Allzyme® Spectrum on the performance of pullets and layers fed low nutrient diet Tuoying Ao*, Anthony Pescatore, Marquisha Paul, Daniel Graugnard, Rebecca Delles, Michael Ford, Ronan Power *Alltech-University of Kentucky Nutritional Research Alliance*

Allzyme® Spectrum is a naturally fermented product with multiple enzyme activity including carbohydrase and phytase. A study was conducted to investigate the effect of dietary Allzyme® Spectrum on the growth performance of pullets, egg production of laying hens and egg and shell quality. Dietary treatments included: 1) corn-soy diet having a nutrient level equivalent to commercial level; 2) corn-soy diet having 0.15% less Ca and available P compared to diet 1 (Low Ca & P diet) + commercial phytase; 3) Low Ca & P diet + 0.02% Allzyme® Spectrum; 4) Low Ca & P diet having 90 kcal/kg less ME + 0.02% Allzyme® Spectrum. Eight replicate cages of eight Hy-line brow pullets were randomly assigned to each of the four dietary treatments from day one and were raised for 16 weeks. Then, the pullets were weighed and transferred to the layer rooms with the same treatment diets. A total of 144 pullets were used in the layer trial with six replicate units of six hens. The layers were housed in layer cages with two birds per cage (25 x 41 cm) and photo-stimulated based on the management guide from Hy-line. Data were subjected to ANOVA for completely randomized design to identify treatment effects and LSD was used to determine mean differences. The body weight and feed intake of pullets were not different among the treatment groups at the end of pre-lay period. The HDP, feed intake, egg weight and shell quality were not different during 20-week of egg production between each of treatment groups. The results in this study indicated that diets with reduced ME, Ca and available P and supplemented with Allzyme® Spectrum can be used to rear pullet and feed layers during peak production without affecting performance, egg weight and shell quality.

Key Words: pullet, Layer, production performance, Enzyme, reduced nutrients

P319 M52, a natural coccidiostat, improved performance, fecal oocyst shedding and intestinal lesion score of Eimeria-infected broilers Hongyu Xue*, San Ching, LeAnn Johnston *Amlan International*

M52 is an all-natural feed additive (Amlan International, Chicago, IL) that features a proprietary blend of select antiprotozoal phytochemicals. Six *in vivo* trials were conducted to evaluate the effects of M52 supplementation on growth performance, fecal oocyst shedding, and intestinal lesion score of broiler chickens challenged with experimental coccidiosis. In these 6 trials, the birds were either challenged with sporulated oocysts of a single *Eimeria maxima* strain or a cocktail of *E. acervulina*, *E. maxima* and *E. tenella* on d14 or 15. Durations of these 6 trials ranged from 20 to

28 days. All the 6 trials included a non-supplemented challenged control and a treatment group receiving supplementation of M52 at 70 g/MT. A meta-analysis was performed to combine the data from all the 6 studies to estimate the common effect of M52 on the performance and health of *Eimeria*-challenged broilers as compared to the challenged control. Data were analyzed by the Random-effects model using version 2.0 Comprehensive Meta-Analysis software, with trial considered 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 M52 at 70 g/MT had significantly greater weight gain (overall: 184 vs 160 g, $P < 0.0001$; post challenge phase: 55.6 vs 50.7 g, $P < 0.005$) and improved FCR (overall: 1.75 vs 1.88, $P < 0.001$; post challenge phase: 2.25 vs 2.73, $P = 0.0001$) than the control birds. M52 treatment also decreased cumulative fecal oocyst count (3.92 vs 4.07 log₁₀, $P < 0.0001$) and reduced coccidial lesion score (1.98 vs 2.55, $P < 0.0001$). In two of these studies, M52 was further tested against multiple most commonly used anticoccidial drugs including chemicals and ionophores. M52 supplementation consistently provided comparable size of benefits to coccidial lesion and feed efficiency in both studies as compared to Salinomycin, Nicarbazine or Maxiban™ (narasin and nicarbazine).

Collectively, M52 has potential as an alternative to ionophores and chemical coccidiostats to help prevent coccidiosis while maintaining gut health and bird productivity.

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

P320 In vitro and in vivo evaluation of A220, as an in-feed antibiotic alternative for enteric pathogen control in poultry and swine Hongyu Xue, Dongping Wang, San Ching, Sara Johnston* *Amlan International*

A220 is an all-natural feed additive (Amlan International, Chicago, IL) featuring a blend of a proprietary toxin-adsorbing mineral with essential oils and medium-chain fatty acids that is optimized for pathogen control in livestock production. The antimicrobial ingredients of A220 have been shown to have a broad-spectrum bacteriostatic/bacteriocidal activity *in vitro* at minimal inhibitory concentrations against *C. perfringens*, *E. coli* O157, *E. coli* K88⁺, *P. multocida*, *S. Enteritidis* and *C. jejuni* at 0.08 to 0.6 mg/ml. Of note, for a variety of common zoonotic pathogens, the virulence is mostly associated with their ability to produce toxins. The enterosorbent mineral component of A220 can also effectively bind various exotoxins produced by *E. coli* (heat-labile and Shiga-like toxins), *C. perfringens* (α - and NetB toxins), and *V. parahaemolyticus* (PirA and PirB toxins).

Effects of A220 on animal health and performance was further examined in various *in vivo* pathogen-challenged experimental models. In two studies using a *C. perfringens*-induced necrotic enteritis model, for broilers fed A220, body weight gain, feed intake, feed conversion ratio, necrotic enteritis-associated mortality and lesion scores were lower ($P < 0.05$) than challenged control (CH) birds and were not different to birds supplemented with BMD ($P > 0.05$) in both experiments. Further, ELISA demonstrated a marked reduction in cecal *C. perfringens* populations and cecal alpha-toxin levels compared to the CH group ($P < 0.05$). In a *S. Heidelberg* (SH) infection model, compared to CH broilers, A220 significantly reduced the prevalence of SH in cecal contents (50% vs. 83.3%, $P < 0.05$). In pigs challenged with an enterotoxigenic *E. coli*, A220 at 0.25% and 0.50% increased post-infection (PI) feed efficiency and reduced diarrhea frequency during the study ($P < 0.05$). A220 also promoted a well-balanced gut microbial ecosystem by selectively increasing the relative abundance of *Firmicutes*, especially that of *Lactobacillaceae*, but reduced *Bacteroidetes* and *Proteobacteria* in feces on d7 PI ($P < 0.05$).

Collectively, A220 has potential for enteric pathogen control in poultry and swine by targeting pathogenic bacteria and their exotoxins, and can be an effective, all-natural alternative to in-feed antibiotics.

Key Words: *C. perfringens*, *S. Heidelberg*, enterotoxigenic *E. coli*, bacterial toxin, antibiotic alternative

P321 Determination of arabinoxylan content in feedstuffs and its prediction by NIRS, to better predict the nutritional value of enzymes carbohydrases Marcio Ceccantini¹, Vincent Larat², Sophie Le-Gall³, Aurelie Preynat² ¹Adisseo France, ²Adisseo France SAS, ³INRAE, BIBS facility, PROBE infrastructure

Carbohydrases are used on poultry diets to release energy and nutrients by countering the adverse effect of non-starch polysaccharides (NSP), but inconsistency maybe found, depending on dietary NSP composition, mainly arabinoxylan (AX). The efficacy of feed enzyme rich in xylanase and arabinofuranosidase is closely linked with the level of AX in the feed (Musigwa et al., 2021). So, the objective of this paper is to assess the AX variability of feedstuff samples collected worldwide and develop Near Infra-Red (NIR) calibrations for determination of water-extractable (WE-AX), water-unextractable AX (WU-AX) and total AX (tot-AX) contents of feedstuffs to adjust the enzyme nutritional value. A total of 364 samples of raw materials were analyzed for their AX contents in WE and WU fractions by gas chromatography after total acid-hydrolysis. Based on the tot-AX, the content varied from 32.8% for corn bran to 3.4% for sorghum. The averaged results of tot-AX are 4.6, 7.2, 11.8 and 24.3% for corn, wheat, corn-DDGS and wheat bran, respectively. The proportion of WU-AX represented from 90 to 98% of the tot-AX. The coefficients of variation of tot-AX observed for corn (n=55), wheat bran (n=42), wheat (n=29), corn-DDGS (n=32) and barley (n=29) are 11.7, 14.0, 11.5, 27.8 and 21.0%, respectively. A larger variability is observed for WE-AX compared to WU-AX. All these data demonstrate a wide variation in AX content between and within raw materials. The same samples were analyzed on a NIR instrument over the wavelength range 1100 – 2500 nm to develop NIR calibrations for AX content prediction. The biochemical measurement of AX contents from 302 samples were used as calibration set while those from 62 samples were used as an independent test set, with similar proportions of given raw materials within each set. The standard error of prediction (SEP) obtained on the set of 62 independent samples was found to be 1.02 % ($R^2 = 0.97$) for both tot- and WU-AX. Focusing on samples having less than 10% tot-AX (N=46) allowed to improve NIR predictions accuracy to reach a SEP of 0.71% ($R^2 = 0.90$) on tot-AX and a SEP of 0.62% ($R^2 = 0.91$) on WU-AX, demonstrating the usefulness of NIR spectroscopy to predict tot and WU-AX contents of these raw materials and therefore adjust enzyme application.

Key Words: arabinoxylan, enzyme, NIRS, NSP, cereals

P322 Evaluation of effectiveness of feed additives to reduce *Salmonella heidelberg* colonization in broilers. Brian Glover¹, Jose Charal¹, Milan Hruby¹, Jennie Baxter², Charles Hofacre² ¹Archer Daniels Midland Animal Nutrition, ²Southern Poultry Research Group, Inc.

The objective of the current study was to evaluate the effectiveness of an inactivated whole *Pichia guilliermondii* yeast product (WYP; Citristim®), along with an organic fatty acid product (SCFA/MCFA; Daafit® Plus) to reduce the colonization of *Salmonella heidelberg* (S.H.) in broiler chickens. In this pen study, 1,800 Ross x Ross chicks were assigned to four treatments, with nine replicate blocks, and allocated into groups of 50 birds per pen. Treatments 2, 3, and 4 were assigned to pens using a randomized complete block design. Treatment 1 consisted of a non-challenged control. Treatment 2 consisted of a challenged control, Treatment 3 was a combination of WYP (0.5lbs/ton) and SCFA/MCFA (1lb/tonne). Treatment 4 consisted of a combination of WYP (1lb/ton) and SCFA/MCFA (2lb/ton). Bird weights by pen were recorded at study initiation (DOT 0), DOT 21, DOT 35, and termination (DOT 43). Seeder birds were one-half of the 50

birds/pen in the study. On DOT 3, 25 seeder chicks per pen were tagged, color-coded (for identification), and 1.1×10^7 CFU nalidixic acid-resistant S.H. via oral gavage. All statistical testing assumed a two-sided alternative hypothesis, and $P < 0.05$ was considered significant. There were no significant body weight differences between treatments. The level of S.H. in the environment, as measured by most probable number (MPN) per bootsock, demonstrated an effect of the feed additives on the numerical reduction untreated (3.12 MPN), lower inclusion (2.57 MPN), and higher inclusion (2.27 MPN). Ten ceca from horizontal (indirect) challenged and five from direct challenged (seeders) were collected from each pen on day 42. There was a reduction in *Salmonella* prevalence of the horizontal challenged by both inclusion rates. The highest inclusion of feed additives had the most significant reduction ($P = 0.013$). There was a numerical reduction in the direct challenged that was greater at the higher inclusion, 51.1% untreated; 42.2% lower inclusion and 37.8% higher inclusion. Overall the combination of WYP and SCFA/MCFA may have contributed to a reduction of the S.H. colonization prevalence in the direct salmonella-challenged broilers.

Key Words: *Salmonella heidelberg*, *Pichia* yeast, *Pichia guilliermondii*, SCFA, MPN

P323 Liquid acidifier via drinking water in broiler performance, a field study Cinta Sol^{*}, Mónica Puyalto, Juan José Mallo NOREL S.A.

The aim of this field study was to evaluate the effect of water acidification with organic acids on broiler performance and cost of medication.

A total of 10 houses with around 30,000 birds per house took part of the study. From the 10 houses, 8 houses were maintained as an unsupplemented treatment (CON, n=8) and 2 houses were supplemented with a liquid acidifier (LCu, n=2) based on sodium butyrate, formic acid, lactic acid, propionic acid and copper, with a total active part of 69.3% (Gustor L Cu, NOREL, SA). The trial lasted 34 days. In one of the LCu houses (LCu-1), the product was dosed at 1kg/1000L of drinking water from day 17 until day 50 (3 days per week during 24 hours). In the other LCu house (LCu-2), the product was dosed at 0.5kg/1000L of drinking water from day 17 until day 50 (during 24 hours per day).

The parameters recorded at the end of the rearing period were, livability, final body weight (FBW), feed conversion ratio (FCR), productivity index (EEF) and cost of medication. The results were analyzed by one-way ANOVA using the GLM procedure of SAS. Means separation was done by Tukey's Test. Only two treatments were taken into account, unsupplemented and supplemented with liquid acidifier.

There were non-significant differences in livability between supplemented and unsupplemented treatments. Regarding FBW, birds in LCu-2 were non-significant 125g heavier than birds unsupplemented. There was a tendency in FCR, (1.65 vs 1.62 for CON and LCu, respectively, $P=0.079$) and in EEF (397.6 vs 414.9 for CON and LCu, respectively $P=0.097$).

Finally, the cost of medication per bird was lower for supplemented birds with respect to unsupplemented. LCu1 group saved 0.0034\$ per bird in medication investments. The total benefit of LCu1 was 0.062\$/bird compared to CON taking into account the cost of the product and the EEF. LCu2 group saved 0.0064\$ per bird in medication investments. The total benefit of LCu1 was 0.16\$/bird compared to CON, taking into account the cost of the product and the EEF.

In summary, the use of liquid acidifier (Gustor L Cu) via drinking water can improve performance parameters in broilers, reduces the cost of medication and increases the profit at the end of the rearing period in commercial conditions.

Key Words: liquid acidifier, broilers, performance, sodium butyrate

P324 Effects of an herbal adaptogen feed-additive on growth performance, carcass parameters, and meat quality in heat-stressed modern broilers Elizabeth Greene, Clay Maynard, Casey Owens, Jean-François Meulenet, Sami Dridi* *University of Arkansas*

Rising global temperatures threaten the sustainability of all agricultural systems, and poultry in particular, due to their high metabolic and growth rates. The adverse consequences of heat stress on poultry production are well recognized, and current research aims to investigate measures to mitigate these negative effects. Here, we sought to evaluate the effects of in-feed herbal adaptogen (stress response modifier) supplementation on growth performances, meat quality, and breast amino acid profile in chronic cyclic heat-stressed broilers. Day-old male Cobb 500 chicks (n=672) were randomly assigned to environmental chambers (n=12, 24 pens), with 3 diet-treatments: a 3-phase corn-soybean based diet fed as such (Control, C), or supplemented with an herbal adaptogen (Natural Remedies, Bangalore, India) at 500g/1000kg control diet (NR-PHY-500) or at 1kg/1000 kg control diet (NR-PHY-1000). From d29 to d42, birds from 9 chambers were exposed to 8h of cyclic heat stress (HS, 35°C from 9:30 am-5:30 pm); whereas the rest of the chambers were maintained at thermoneutral conditions (24°C, TN), thereby providing 4 experimental groups: C-TN, C-HS, NR-PHY-500HS, and NR-PHY-1000HS (6 pens/group, 168 birds/group). HS altered growth performance via reductions in feed intake and body weight as compared to C-TN ($P < 0.05$). Adaptogen supplementation stimulated feed intake, increased body weight, and resulted in 5 and 10 points better FCR at the low and high dose, respectively, compared to the C-HS group. This increase in body weight was reflected in enhanced weights of body parts (breast, tender, wings, and legs), which was significantly different from C-HS with NR-PHY-1000HS ($P < 0.0001$). Additionally, NR-PHY-1000HS decreased ($P < 0.01$) both the incidence and severity of woody breast, as compared to other treatments. Adaptogen supplementation also influenced meat quality, through modulation of the breast amino acid profile, pH, color, cook loss, and tenderness. Together, these data suggest that adaptogen supplementation could be a promising solution to alleviate heat stress, however further in-depth investigation of its mode of action and its underlying mechanisms are necessary.

Key Words: adaptogen, broiler, heat stress

P325 Measurement of digesta peptidoglycan depolymerization induced by muramidase using a LCMS-MS method Jacqueline de Souza¹, Mads Pedersen², Carsten Frederiksen², Estefania Calvo³, Rual Lopez-Ulibarri³, Mikkel Klausen², Diogo Rosso¹ ¹*Novozymes Latin America Ltda*, ²*Novozymes A/S*, ³*DSM Nutritional Products*

Besides the well-known feed enzyme categories such as phytases, proteases and carbohydrases, other enzyme classes can also be evaluated for animal performance improvement by targeting different components present in the gastro intestinal tract than those originating from the ingested feed. Microbiota in the gastrointestinal tract of animals lives in homeostatic equilibrium with the host. Natural death and cell turnover of the microbiota causes the release of cell components or bacterial cell debris to the gut environment that could interact with the intestinal wall and impact gastrointestinal functionality. Analytical tools to study bacterial waste are needed to understand how dead bacteria and cell debris can impact *in vivo* performance. A major part of the bacterial biomass is peptidoglycan, a complex polymer forming the bacterial cell wall, containing short peptide bridges, Nacetylglucosamine and Nacetylmuramic acid, the latter of which is uniquely found in peptidoglycans and, therefore, can be used as a bacterial waste biomarker. Recent studies reported how *in vivo* supplementation of a novel fungal muramidase enzyme, capable of depolymerizing peptidoglycan from dead bacteria, significantly improved performance parameters in broiler chickens. A LCMS-MS method was developed to quantify the concentration of total and soluble muramic acid after acid hydrolysis in digesta samples. The total concentration of muramic acid in digesta increased through the gastro intestinal tract with the highest concentration observed in caecum. A significant increase in the

ratio between the soluble and total muramic acid concentration was observed by muramidase supplementation (45.000 LSU/kg) in all analyzed segments; crop, jejunum and caecum ($P < 0.05$). The effect of muramidase supplementation was most pronounced in jejunum with approximately twice as high soluble-to-total muramic acid ratio compared to the control. In addition, a significant effect of muramidase dosage on the soluble-to-total muramic acid ratio was observed when supplementing three different dosages of muramidase (25.000, 35.000, and 45.000 LSU/kg) in ileum collected samples ($P < 0.05$), emphasizing the small intestine as a key segment associated with muramidase performance and effect *in vivo*.

Key Words: Muramidase, Enzyme, Gut health

P327 Effect of a proprietary plant extract-based product on body weight, weight gain, and feed conversion when broilers received a coccidiosis vaccination without or with a chemical bioshuttle program Muhammed Shameer Abdul Rasheed*, Mark LaVorgna, Kristy Dorton *Devenish Nutrition, LLC*

Two studies were conducted to determine the effects of a proprietary plant extract-based product (EO; DeviSTAT Broiler NA, Devenish Nutrition, Fairmont, MN) on body weight, weight gain, and feed conversion when broilers received a coccidiosis vaccine without or with a chemical bioshuttle-

tle program. In each study, 2,040 broiler chicks (1-day old Cobb 500 broilers, as hatched) were randomly assigned to 24 pens (85 birds per pen; 0.90 ft²/bird). Each study included four diet phases: starter (d 0 to 14), grower (d 15 to 28), finisher (d 29 to 38) and withdrawal (d 39 to 49). Birds were fed a corn-soy based pelleted diet, which contained the study treatments. In study 1 (49 d), there were two treatments: Control (basal diet, no test product) or EO at 0.75 lb/ton in the starter and grower diets only. No coccidiostat program was added to the diets in study 1. In study 2 (48 d), the two treatments were: Control (basal diet, no test product) or EO at 0.75, 0.50 and 0.75 lb/ton in the starter, grower, and finisher diets, respectively. Birds in study 2 also received a coccidiostat (113.5 g/ton Zoalene) in the grower diet. In both studies, all birds received a coccidiosis vaccine (HatchPak, Merial, Gainesville, GA) at placement (d 0). Body weight was measured at d 0, 14, and 48 or 49. Weight gain and feed conversion ratio (FCR) were determined from d 0 to 14, d 15 to 48 or 49, and d 0 to 48 or 49. Data was subjected to a one-way ANOVA using the Mixed procedure of SAS 9.4 (2018) with pen as the experimental unit. Means were separated by Fisher's protected least significant difference. Differences were considered significant at $P \leq 0.05$. Body weight and weight gain were not significantly affected by treatment in either study. In study 1, FCR was better in broilers supplemented with EO than broilers fed the control diet from d 15 to 49 ($P = 0.002$; 1.928 vs. 1.989) and d 0 to 49 ($P < 0.001$; 1.861 vs. 1.919). In study 2, FCR was better for broilers supplemented with EO from d 0 to 15 ($P = 0.03$; 1.320 vs. 1.374) and d 0 to 48 ($P = 0.05$; 1.685 vs. 1.708). Results show that supplementation of a proprietary essential oil product improved FCR by 5.8 and 2.3 points, respectively, when broilers received a coccidiosis vaccine alone or in combination with a chemical bioshuttle.

Key Words: Broiler, Plant extract, Coccidiosis vaccine, Chemical bioshuttle, Feed conversion

P328 Low density feed ingredient and supplemental enzymes as nutrition strategy for economic broiler production in Egypt Nesslerin Selim^{*1}, Mona Mostafa², Hemat Abdel Magied¹, Heba Habib¹, Mohamed El Menawey³ ¹*Department of Poultry Nutrition Research, APRI, ARC,* ²*Department of Poultry Breeding Research, APRI, ARC,* ³*Department of Animal Production, Faculty of Agriculture, Cairo Univ.*

This trial was conducted to examine a nutrition strategy to decrease broiler feed cost by using low density ingredient during starter (St) and grower (Gr) periods supplemented with enzymes mixture (E). Corn/soy diets (C) were formulated to provide Arbor Acres broiler chicks with all nutrients requirements. Wheat bran was used as diet diluent at level of 10% (w:w), while during finishing (F) period all chicks received C finisher diet. Compared to C diets, the diluted diet (DD) contained 165 kcal/kg, 1%, and 0.08% less ME, CP, and Ca during St and less 177kcal/kg, 0.8%, and 0.08% during Gr, but values of av. P and CF increased by 0.01 and 0.7% during both phases. One hundred and fifty day-old broiler chicks were distributed into 5 treatments of 3 replicates of 10 chicks of each. The five treatments were: 1, (C): control; 2, (C250E): C+250 g E (E included 5000 IU Xylanase, 10000 IU Cellulase, 7500IU Amylase, 5500 IU Protease, 1000 FTU Phytase, 800 IU B Glucanase, and 900 IU Pectinase/g); 3, (C500E): C+500 g E; 4, DD; 5, (DD500E): DD+ 500 g E. The experiment extended till 42 days of age. Growth performance, and carcass traits were recorded, and economic efficiency of all treatments were calculated. Prices of St and Gr DD500E were 4.2% and 3.9% cheaper than those of C diets. C500E showed best performance during St and Gr phases, while DD500E showed best values during both F and overall periods. DD500E showed best significant overall FCR (1.45) compared with all other treatments (vs 1.52 to 1.57 for other groups). The same trend was recorded for dressing% (81.6 % vs 72.3 - 74.4%), wing's weight % (3.89% vs 2.96 - 3.33%), tibia weight% (1.82% vs 1.0 - 1.23%) and tibia length (12.1 vs 10.17-10.83 cm). While measurements of carcass, intestine, caeca, and caeca pH showed only numerical improvements with DD500E birds. The same strategy showed decreased feed cost/kg of body weight (9.6 vs. 10.55 LE=0.06\$), and increased net revenue/bird compared to C (10.15 vs. 5.8 LE=0.28\$), further more C250E and C500E showed 7.6 and 8.5 LE net revenue/bird. However, applying DD500E strategy during St and Gr phases showed increase of revenue by 2800\$/10000 birds/cycle, more research is needed about the effects on gut health.

Key Words: Broiler, enzymes, growth performance, feed cost

P330 Effect of conditioning temperature and retention time on pellet durability index Leopoldo M. Almeida^{*1GS}, Susan M. Bonilla², Joseph P. Gulizia², José I. Vargas², José R. Hernandez², Wilmer J. Pacheco²

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Thermal process during conditioning and pelleting typically increases quality pellet, which can lead to improvements on broiler performance in comparison to mash diets. However, there is limited research evaluating the effect of retention time on pellet quality. The objective of this study was to evaluate the effect of conditioning temperature and retention time on pellet quality. The experiment consisted of a 2 x 3 factorial arrangement of 2 conditioning temperatures (82 and 88°C) and 3 retention times in the hygieniser (40, 80 and 120 seconds). Corn-soybean meal-based diets with 3.33% soybean oil addition were mixed in a twin shaft mixer and steam conditioned and pelleted using a constant production rate of 0.7 ton/h with 50-horsepower pellet mill. Pellets samples were collected throughout the run and immediately cooled. 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 30 seconds. Data were statistically evaluated using GLM procedure of R and means were separated by Tukey's HSD with statistical significance considered at $P \leq 0.05$. There was no interaction between conditioning temperature and retention time on PDI ($P > 0.05$) for both methodologies. Increasing conditioning temperature improved ($P < 0.05$) PDI from 73.17 to 77.32% and 68.52 to 74.31% when measured with the ASABE method S269.5 and Holmen NHP 100 tester, respectively. Increasing conditioning temperature from 82 to 88°C improved PDI, but the retention time in the hygieniser did not have an effect on PDI ($P > 0.05$) regardless of the methodology, which suggest that 40 seconds retention time is enough for moisture and heat migration to the center of the feed particles.

Key Words: Feed form, hygieniser, pellet quality

P331 New generation high protein dried distillers grain in White Leghorn laying hens diets Josephine Foley^{*1GS}, Sheila Purdum¹, Mallorie Wilken² ¹University of Nebraska - Lincoln, ²ICM, Inc

As a feed product of ethanol production, Dried Distillers Grains have become a popular protein source in poultry rations. This study investigated the effects of two new generation Hi-Pro DDG: FST1 Hi-Pro DDG contained 40.3% protein and FST2 Hi-Pro DDG contained 39.1 % protein, on feed intake, egg production parameters, and apparent metabolizable energy (AME) in laying hens. The DDGs for this study were sourced from ICM, Inc. A total of 216 Shaver White Leghorn laying hens were housed in 63 cages from 21 to 45 weeks of age. Each cage was assigned to one of 7 treatment groups with 9 replicate cages in a randomized complete block design. Treatment 1 was a corn/soy control with no DDG in the diet. Treatments 2-7 were a factorial combination of the 2 DDG sources fed at 3 inclusion levels, (5, 10 or 15%) in the rations. Egg production (EP), feed intake (FI), and mortality were recorded daily. Egg weights (EW) and yolk color score were measured biweekly. Hen weights and eggshell breaking strength were measured monthly. Manure was collected and analyzed for apparent metabolizable energy determination at the end of the study. Titanium dioxide was utilized as an inert marker in the AME determinations. Significant time x dietary treatment interactions were found for EP ($p < 0.02$), yolk color score ($p < 0.003$) and eggshell breaking strength ($p < 0.07$). EP improved during the later weeks of the trial in hens fed the diets either DDG source at 15% of the ration. Yolk color score increased as DDG level increased in the diet but did show some decrease over time regardless of treatment. Eggshell breaking strength was higher in the control and 15% FST2 DDG treatments ($p < 0.195$). DDG source or level did not significantly affect FI, EW, or hen wts. Diet had a significant effect on apparent metabolizable energy with an improvement measured for 5% FST1 or 10% FST2 treatment groups and for all hens fed DDG compared to the control group ($p < 0.0001$). In summary, hens could be safely fed up

to 15% of the new generation FSTTM Hi-Pro DDG with no detrimental effects on FI or EP in laying hens and with some improvements to yolk color and EP shown in later stages of EP.

Key Words: Laying hen, DDG, High protein DDG

P334 Effect of Poor-Quality Soybean Meal on Broiler Performance

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Variations in soybean meal (SBM) quality can be caused by uneven cooking, undercooking, and overcooking which can result in antinutritional factors such as trypsin inhibitors and low-quality diets. The objective of this study was to determine if poor quality SBM would negatively impact growth performance and enteric permeability including bacterial translocation (BT), body weight (BW), and serum fluorescein isothiocyanate dextran (FITC-d) in broilers. To produce poor-quality SBM (pqSBM), a 1:1:1 mixture of raw SBM, normal SBM, and overcooked SBM replaced normal SBM in the treatment diets. Overcooked SBM was created by autoclaving SBM for 120 min. The trypsin inhibitor activity of pqSBM was 5.9mg/g, indicating a sufficient quantity of antinutritional factors to impair protein digestibility. The trial consisted of 3 treatments, Control, Rye + Meat and Bone Meal (MBM) + pqSBM, and MBM + pqSBM. Each treatment consisted of 4 replicate pens with 50 birds per pen (n = 200/trt). On day of hatch (DOH), *E. cecorum* (1 x 10⁴ CFU/chick) was administered to all birds by oral gavage, and 15 birds per pen tagged to monitor BW throughout the experiment. On DOH, d15 and d35, tagged birds were weighed, and on d15 and d35, 3 randomly selected birds from each pen were orally administered FITC-d (4.17 mg/kg) 2h prior to sample collection. Free thoracic vertebrae (FTV) were aseptically collected for plating on TSA and CHROMagarTM to assess BT and presence of *Enterococcus* spp., and blood was collected from the femoral artery to quantify serum FITC-d recovery. All data were analyzed via ANOVA and significant differences amongst means were determined using Tukey's HSD test (p<0.05). There were no differences for FITC-d recovery and BT at either time point. However, there were significant decreases in BW (p<0.001) of birds fed diets containing pqSBM. There was also a significantly higher level of *Enterococcus* spp. (p<0.001) in the FTV of birds fed pqSBM at d15, but no difference at d35. Based on these results, pqSBM suppresses growth performance while also predisposing birds for increased translocation of *Enterococcus* spp. early in life. These results highlight that poor diet quality could predispose birds to opportunistic diseases that result in economic losses beyond lowered BW.

Key Words: Soybean Meal, Bacterial Translocation, Broilers, Performance, Enterococcus

P335 An assessment of feed color effects on broiler performance parameters during the starter phase

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A 21-d study was conducted to assess the implications of coloring a broiler starter diet either blue or purple on bird performance and feed color preferences. Feed color treatments were created using a common broiler starter

diet (crumbled) either dyed blue, purple, or undyed (basal diet). Cobb 500 byproduct males (216) were randomly allotted to 18 battery cage units (12 birds/cage, 6 replicates/treatment). Two feed troughs were affixed to each cage allowing assignment of feed color treatments as: (1) basal-blue, (2) basal-purple, or (3) blue-purple. Birds assigned to each cage had *ad libitum* access to feed and water throughout the study. Bird weights and feed consumption (FC) data were collected at 7 d intervals. Data were analyzed as a completely randomized design using the GLM procedure of SAS. Means were separated using Tukey's HSD and statistical significance considered at $P \leq 0.05$. With color combinations taken together, there was little influence on bird performance. Birds offered the basal-purple combination had a 3.8% higher ($P=0.045$) d 21 body weight than those on the basal-blue treatment. Interestingly, birds given the blue-purple feed combination had the lowest feed conversion between d 0 and 14 ($P=0.045$) and 0 and 21 ($P=0.101$). When FC assessed by trough was compared, a clear preference for the basal diet was elucidated. Day 0 to 14 and 0 to 21 FC was 27.6 and 28.7% lower for purple than the basal diet, respectively. Likewise, blue diet consumption tended ($P=0.098$) to be lower (18.3%) than basal between d 0 and 14. When given the choice between a basal diet and either blue or purple, birds appeared to have a more substantive aversion to purple in this study. Feed consumption for all periods did not differ when birds were given the choice between only blue or purple feed. When both feed colors were grouped and contrasted with basal, d 0 to 21 FC was 18.3% higher ($P=0.001$) for the basal diets. Based on these results, there appears no benefit to the coloring of broiler feed blue or purple during early growout. Further, when given the choice between a blue or purple diet and a typical non-colored diet, birds appeared to have an aversion to the colored feed based on differences in feed consumption data.

Key Words: Broilers, Feed color

P336 Net energy concentration of soybean meal for broiler chickens using the comparative slaughter technique

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The objective of this study was to determine the net energy (NE) concentration in soybean meal (SBM) fed to broiler chickens using the comparative slaughter technique. Two different types of SBM containing 460 g/kg crude protein (SBM-46) and 480 g/kg crude protein (SBM-48) were used in the current study. On d 17 post hatching, 108 male broiler chickens were fasted for 12 h. On d 18 post hatching, birds were individually weighed (initial body weight = 701 ± 68 g) and divided into the initial (8 birds) and final group (96 birds). The initial group was euthanized by CO₂ asphyxiation and immediately frozen. Remaining 96 birds were assigned to 3 experimental diets with 4 birds per cage in a randomized complete block design with body weight as a blocking factor. Experimental diets consisted of a basal diet and 2 test diets in which 20% of SBM-46 or SBM-48 replaced the energy-yielding ingredients in the basal diet. The experiment lasted for 7 d and excreta collection was conducted during the last 3 d. On d 25 of age, birds were euthanized by CO₂ asphyxiation after a 12 h-fast, individually weighed, and immediately frozen. Whole frozen carcasses in the same cage were ground together, thoroughly mixed, and dried at 55°C for gross energy analysis. Nitrogen-corrected metabolizable energy (ME_n), energy retention, and NE in the basal diet were greater ($P < 0.05$) than values in the test diets containing SBM-46 and SBM-48. No difference was observed in ME_n between the SBM-46 and SBM-48 diets, but NE in the SBM-46 diet was greater ($P < 0.05$) than those in the SBM-48 diet. On a dry matter (DM) basis, ME_n in SBM-46 (2,835 kcal/kg DM) and SBM-48 (2,697 kcal/kg DM) did not differ, whereas NE was greater ($P < 0.05$) in SBM-46 (2,094 kcal/kg DM) compared with SBM-48 (1,241 kcal/kg DM). In conclusion, NE concentration in SBM with low protein was greater than that in SBM with high protein.

Key Words: broiler, comparative slaughter, metabolizable energy, net energy, soybean meal

P337 Effect of protein level and grain damage on carbohydrate, crude protein, amino acids, and trypsin inhibitor content of soybeans.

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Soybean is the main source of crude protein (CP) and amino acids (AA) for poultry diets. However, the impact of bean damage on the nutrient and antinutrient content has not been well explored. This study aimed to determine the effects of raw soybean quality within four kernel damage categories (as is, good, purple damage, and completely damaged) and two protein levels (41.5 and 45.6% DM) on three α -galactosides, sucrose, non-starch polysaccharides (NSP), CP, AA, and trypsin inhibitor (TI) content. Soybeans from two CP levels were planted and harvested under similar conditions and classified into four quality categories based on kernel damage. NSP content, including solubility fractions, was determined by GC analysis of constituent sugars, while sugars and oligosaccharides were determined by ion chromatography. Other compounds CP, AA, and trypsin inhibitor content, were determined by NIRS. Data were analyzed using a two-way ANOVA in a completely randomized design. Interaction effects ($P < 0.05$) between protein level and bean quality were observed in all parameters evaluated, except for verbascose and methionine content. Regardless of the protein level, completely damaged kernels presented the lowest ($P < 0.05$) values for raffinose, stachyose, total α -galactosides, and sucrose compared to the good quality beans. In contrast, completely damaged soybeans presented the highest values ($P < 0.05$) for soluble, insoluble, and total NSP at both protein levels compared to soybeans from other quality categories. CP and AA were reduced as grain damage increased ($P < 0.05$). Still, 41.5% of CP kernels resulted in lower nutrient content than their counterpart at 45.6%. Additionally, the lowest TI was detected in kernels from the as-is group at 41.5%, while good and purple beans indicated the highest values. Intermediate responses were observed in beans from all categories at 45.6% CP. Kernels with 41.5% of CP resulted in 0.6 g more ($P < 0.05$) soluble NSP compared to 45.6% CP grains. The damaged group consistently demonstrated the lowest ($P < 0.05$) α -galactosides, sucrose, CP, AA, trypsin inhibitor, and soluble NSP, but showed the greatest content of total NSP. In conclusion, kernel quality affected carbohydrate, CP, AA, and trypsin inhibitors in soybeans.

Key Words: Soybean, carbohydrates, trypsin inhibitor, kernel damage, soybean quality

P338 Effects of mix time on coefficient of variation (mix uniformity) and broiler growth performance

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Previous research has reported that mixing uniformity is inversely proportional to mixing time. The majority of feed manufacturers use the same mixing time throughout the growing period regardless of age and feeding phase. However, throughout this period, birds may require a more or less uniform mix depending on consumption patterns. The objective of this study was to evaluate the effects of mix uniformity on broiler growth performance. A total of 640 male Ross×Ross 308 broiler chicks were randomly distributed in 40 floor pens with 10 replicate pens/treatment and 16 birds/pen. Feed was manufactured at the North Carolina State University Feed Mill Education Unit utilizing a 2 Ton counterpoise ribbon mixer. Two batches of feed were mixed for 4.5 min (3 min dry mix and 90 s of wet mix) and 30 s (0 s dry mix and 30 s wet mix) to obtain a Uniform (UM) and a Non-uniform (NUM) mix, respectively. Each pen was randomly assigned to 1 of 4 dietary treatments: 1) UM from 1-42 d, 2) UM from 1-28 d and NUM from 28-42 d, 3) UM from 1-14 d and NUM from 14-42 d, and 4) NUM from 1-42 d. Experimental diets were provided in mash form to prevent further mixing of the feed. Ten samples were collected at equally spaced time intervals from the mixer discharge conveyor after each mixing period and analyzed for coefficient of variation (CV) using multiple tracers (chloride ion concentration using Quantab Test Strips,

Microtracer F-Red#40(count), and Microtracer F-Blue#40(count)). Data were statistically evaluated using ANOVA and means were separated by Tukey's honestly significant difference. The experimental design consisted of a 1-way treatment structure using a randomized complete block design with pen location being the blocking factor. There were no statistical differences between the treatments on BW, FI, and FCR during the starter period ($P > 0.05$). In addition, mix uniformity did not influence individual bird BW CV from 1 to 14 d of age ($P > 0.05$). The results of this experiment indicate that mix uniformity may not influence growth performance during the starter period. However, mix uniformity may have an impact on growth performance in subsequent phases.

Key Words: coefficient of variation, mix, broiler, performance, uniformity

P341 Substitution of soybean meal using palm kernel meal and hydrolyzed palm kernel meal supplemented with endo-1,4- β -mannanase enzyme on performance and carcass traits of broilers
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Palm kernel meal (PKM) has potential to be used as dietary protein source for soybean meal substitution in broiler diets. However, high mannan content limits PKM utilization in feed formulation due to low digestibility. This study evaluated the effect of endo-1,4- β -mannanase enzyme on performance and carcass traits of broilers fed palm kernel meal and hydrolyzed palm kernel meal (HPKM). A total of 1,050 day-old chicks were allocated into six treatments with five replicates ($n = 35$). Starter phase: 1) CON (control); 2) PKM1 (1%); 3) HPKM1 (1%); 4) PKM2 (3%); 5) HPKM2 (3%); and 6) COMB (3% PKM + 3% HPKM). Grower phase: 1) CON (control); 2) PKM1 (2%); 3) HPKM1 (2%); 4) PKM2 (7%); 5) HPKM2 (7%); and 6) COMB (3.5% PKM + 3.5% HPKM). Finisher phase: 1) CON (control); 2) PKM1 (3%); 3) HPKM1 (3%); 4) PKM2 (10%); 5) HPKM2 (10%); and 6) COMB (5% PKM + 5% HPKM). Supplementation of endo-1,4- β -mannanase enzyme was 2 g/kg on PKM1 and HPKM1, and 3 g/kg on PKM2, PKH2, and COMB. The results showed that PKM and HPKM supplemented endo-1,4- β -mannanase enzyme did not influence feed intake (FI), BW, or feed conversion ratio (FCR) in the starter and grower phase. Dietary PKM and HPKM decreased BW compared with control ($P < 0.001$), although FI and FCR did not differ in the finisher phase. In addition, higher inclusions of PKM and HPKM (e.g., PKM2, HPKM2, and COMB) had lower slaughterhouse weight ($P = 0.002$), carcass yield ($P < 0.001$), breast yield ($P = 0.02$), and breast pH ($P < 0.05$) compared with CON, PKM1, and HPKM2. Dietary PKM and HPKM also tended to reduce meat tenderness ($P = 0.060$), but did not affect meat cooking loss or water holding capacity. It can be concluded that substitution of PKM and HPKM supplemented with endo-1,4- β -mannanase enzyme have opportunity for broiler diet formulation, but higher levels may reduce broiler performance and efficiency.

Key Words: protein, diet, broiler, performance, mannan

P342 Trends in mycotoxin contamination in 2021 United States corn
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Mycotoxins are secondary fungal metabolites that are detrimental to animal health and a key concern to global food and feed safety. The classic signs such as reduced feed intake and oral and intestinal lesions used as indicators of exposure often underestimate other costs of mycotoxicosis, including increased frequency and severity of disease via immunosuppression, inflammation, and modulation of the gastrointestinal environment. The objective of this study was to evaluate mycotoxin levels in corn from US 2021 harvest and compare those with previous years. Samples of US corn were analyzed utilizing liquid chromatography and tandem mass spectrometry (LC-MS/MS) for six major mycotoxin groups: aflatoxins (Afla), type A trichothecenes (A-Trich), type B trichothecenes (B-Trich),

fumonisin (FUM), zearalenone (ZEN), and ochratoxin A (OTA). The 2021 data were compared to the prior two harvest years using GLIMMIX procedure of SAS with harvest year as fixed effect and sample as the experimental unit with significance reported at $P \leq 0.05$. A limited number of samples are available thus far ($n = 44$) as harvest is still progressing, therefore the risk profile of this crop year is likely to change as the sample pool expands. To date, 100% of samples evaluated contained at least one mycotoxin, compared to 87% observed in 2020. Co-occurrence in corn thus far is comparable with 2020 (2021: 45.4%, 2020: 49%). Currently, FUM is the most prevalent mycotoxin group (2021: 80%; 2020: 65%) with levels significantly ($P < 0.05$) higher in 2021 than 2019 and 2020. Prevalence of B-Trich and ZEN are numerically decreased in 2021 compared to 2020 data (B-Trich: 59% vs. 50%; ZEN: 20% vs. 11%), while Afla prevalence has numerically increased (5% vs. 23%). Contamination levels in corn for Afla, A-Trich, B-Trich, ZEN, and OTA have remained consistent over the survey period with no statistical differences observed. As the mycotoxin risk of this harvest season is still coming into focus, preliminary results of the 2021 corn crop survey indicate continued risk of multi-mycotoxin contamination. As occurrence and contamination levels are similar or higher (FUM) than the prior crop year, mycotoxins may continue to challenge animal health and performance as these ingredients are fed out.

Key Words: Corn, Feed Ingredients, Mycotoxins, Temporal, United States

P343 Full fat high oleic acid SBM in layer hen diets improved monounsaturated fatty acid profile in adipogenic tissue and in egg
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A study was conducted to understand utilization of high-oleic soybeans to produce full-fat, high-oleic soybean meal for use as a potential feed ingredient in layer hen diets for added nutritional value in eggs. Forty-eight layers (~36-week-old) were randomly assigned to one of 4 isonitrogenous (18% crude protein) treatment diets (1-4) with 12 replicate birds/treatment in a 3-week study as follows: (1) Solvent extracted defatted soybean meal + corn diet, (2) dry extruded defatted soybean meal + corn, (3) full-fat soybean meal + corn, (4) high-oleic full-fat soybean meal + corn diet. Apparent ileal digestibility of crude fat (CF) and crude protein (CP) were studied using celite (~2%) as an indigestible marker. Tibia strength and other egg quality parameters (egg weight, shell strength, Haugh unit, etc.) were recorded during the study. Enrichment of monounsaturated fatty acid, oleic acid (C18:1, cis), in adipogenic tissue (fat pad, liver, and breast muscle) and in eggs were measured using gas chromatography (GC-FID). Digestibility values of CF ranged from 75-84% and CP varied from 67-72% for treatment diets, with treatment mean values being no different ($P > 0.05$) between treatment diets. No differences between treatment diets for tibia strength, egg weight, shell strength or Haugh unit were observed except for yolk color. Similarly, there were no difference in the total fats in fat pads, liver, muscle, and egg yolk ($P > 0.05$) between treatment diets. However, the oleic acid % of total fat for treatment 4 diet in fat pads, liver, muscle, and in egg yolk were 45.73%, 47.53%, 31.74%, and 50.68%, respectively, and were significantly higher ($P \leq 0.003$) than other treatment diets. No difference was observed in oleic acid % of total fat in the adipogenic tissue or eggs in other three treatment diets, 1-3 ($P > 0.05$). The results strongly showed that adipogenic tissue and egg quality in terms of oleic acid profile was significantly improved with full fat high oleic acid diet while its digestibility for CF or CP was similar as defatted SBM or full fat SBM diets.

Key Words: oleic acid SBM, digestibility, adipogenic tissue, monounsaturated fatty acid, egg

Metabolism and Nutrition: Vitamins and Minerals

P344 Improvement of egg shell parameters in broiler breeders fed diets supplemented with natural 1,25(OH)₂D₃-glycosides Željko Gottstein¹, Liča Lozica¹, Danijela Horvatek Tomić¹, Josip Miljković¹, Jasna Aladrović¹, Ana Shek Vugrovecić¹, Dalibor Bedeković², Kathrin Bühler³ ¹*Faculty of Veterinary Medicine, University of Zagreb*, ²*Faculty of Agriculture, University of Zagreb*, ³*Herbonis Animal Health GmbH*

High quality eggshell protects the egg from damage and ensures a safe environment for the embryo. Important factors for eggshell formation are calcium and vitamin D. Vitamin D needs to be converted in two steps in the liver and kidney, respectively, into its active metabolite 1,25-dihydroxycholecalciferol (1,25(OH)₂D₃). The conversion is affected by factors that negatively influence these organs, such as mycotoxins and age. In a field trial, the effects of natural 1,25(OH)₂D₃-glycosides from waxy-leaf night shade (*Solanum glaucophyllum*) on eggshell quality in broiler breeders was assessed from 55 to 61 weeks of age. A total of 10'000 Ross 308 breeders in two flocks received either one of two dietary treatments: A standard feed according to breeder recommendation as such (CON) or supplemented with 1 µg of 1,25(OH)₂D₃-equivalents (SG). Dietary vitamin D content in both treatments was 3200 IU. 30 eggs per treatment were collected on experimental day 14, 25 and 35, each, and eggshell quality was assessed. Data were compared between treatments per time point and within treatment over time using LSD-model (Statistica 13). Shell strength in the SG group was 7.20 N and 12.74 N higher, respectively ($p < 0.05$) than in CON at D14 and D35, but comparable ($p > 0.05$) at D25. In CON, shell strength was higher on D25 compared to D14 and D35 ($p < 0.05$) but there was no time effect in SG ($p > 0.05$). Despite the higher breaking strength, eggshell weight did not differ between groups except for D14 ($p < 0.05$). Eggshell thickness deteriorated by 16.5 % in CON between D25 and D35 ($p < 0.05$), but remained constant in SG ($p > 0.05$). This led to a significantly higher eggshell thickness in SG than in CON at D35. There was however no difference in egg weight, neither between treatments nor among sampling times ($p > 0.05$). Egg shape index at D35 was highest in SG (78.3 ± 2.7 %) and lowest in CON (73.3 ± 7.3 %) compared to the other time points within the same treatment ($p < 0.05$). At D35, this parameter was also significantly different between SG and CON ($p < 0.05$). Results showed that adding a natural source of the metabolic active form of vitamin D during 5 weeks mitigated adverse effects of age on eggshell quality and probably increased eggshell stability by changing egg shape.

Key Words: *Solanum glaucophyllum*, vitamin D metabolism, calcium, eggshell, aging

P345 Effect of corn- or wheat-based diets and different levels of non-phytate phosphorus on the quality parameters of fresh and stored eggs Richard Adefioye*, Michael F ord, Anthony P escatore, Harold Gillespie, Jacqueline Jacob, Sunday Adedokun *University of Kentucky*

The objective of this study was to determine the effects of cereal type and dietary level of non-phytate phosphorus (nPP) on the quality of fresh and stored eggs produced by 24-week-old laying hens. This experiment used 504 pullets in a randomized complete block design consisting of 6 treatments with 7 replicates and 12 birds per replicate in a factorial arrangement of treatments involving 2 cereal types (corn-SBM and corn-wheat-SBM) and 3 nPP levels (90, 100, or 110% of requirement). The daily lighting schedule of the birds featured 22 hours of light for the first two weeks, followed by 8 hours of light from weeks 3-16, 12 hours of light by week 17, and a 1-hour weekly increment in light length until week 21 when the birds were exposed to 16 hours of light per day until the end of the study. All birds were fed a standard corn-SBM-based diet that met their nutrient and energy requirements from day 0 to week 12 of age, after which they were assigned to experimental diets. By week 20, the birds were introduced to a new set of experimental diets and they remained on this diet till week 24 of age. Two sets of eggs (6 per replicate cage) were collected by week 24 of age, from which one set was analyzed within 48

hours, while the other was analyzed after 45 days of storage in a refrigerator at 4 °C. The data were analyzed using the appropriate GLM procedure of SAS. No main or interactive effects of cereal type and storage length were observed on egg weight, eggshell breaking strength, eggshell weight, and yolk weight. Storage length affected yolk color, albumen height, and haugh unit, as the stored eggs had lower ($P < 0.05$) albumen height and Haugh unit, but a deeper yellow color compared to the fresh eggs. Diet also affected the yolk color, as the corn-SBM-based diets produced a deeper ($P < 0.05$) yellow-colored yolk compared to the corn-wheat-SBM diet (3.8 vs. 2.3, respectively). Based on the results of this study, a 10 percent reduction or increase in dietary nPP did not affect the egg quality parameters considered. Furthermore, the storage of eggs at 4 °C for 45 days affected the yolk color, albumen height, and Haugh unit.

Key Words: Layers, non-phytate phosphorus, nPP, wheat, storage

P346 Impact of aragonite as an alternative calcium source on hen egg production and egg quality Alyssa Lyons*^{GS}, Logan Erb, Courtney Poholsky, John Boney *Pennsylvania State University*

Limestone is commonly used to provide dietary calcium (Ca) to laying hens. Aragonite is an alternative Ca source made of seashells mined from the ocean floor and may offer egg quality benefits. The objective of the study was to determine the effects of aragonite inclusion on post-peak laying hen egg production and egg quality. A total of 144 40-week-old Hy-Line W-36 hens were housed in cages with three birds per cage. The experimental unit (EU) was two cages of hens with 8 replicates per treatment. The experimental period was 24 weeks (40-64 weeks of age) where hens were fed one of three dietary treatments: Ca provided as a 50:50 ratio of finely ground limestone and limestone chips (PC), Ca provided as a 50:50 ratio of finely ground limestone and aragonite chips (AC), and Ca provided as finely ground aragonite (FA). Each EU contained six hens that were control-fed 600 grams of feed per day. At 52 and 64 weeks of age, one bird per EU was randomly selected, weighed, euthanized, and the left tibia was extracted for bone breaking strength analysis. Measured egg quality parameters consisted of egg weight, albumen height, Haugh unit, specific gravity, eggshell thickness, and breaking strength. The MIXED procedure of SAS was used to analyze egg production data using a repeated measures model. The GLM procedure of SAS was used to conduct ANOVA on egg quality and bone breaking strength parameters. Overall, Ca source did not affect egg production from 40-64 weeks of age ($P > 0.05$). As expected, egg production declined from week 52 to 64 ($P < 0.05$). At 52 weeks of age, albumen height and Haugh unit were highest for hens fed AC ($P < 0.05$) and egg breaking strength was highest for hens fed FA ($P = 0.003$). At 64 weeks of age, specific gravity was highest for hens fed the FA and lowest for hens fed the PC diet ($P < 0.05$). Neither egg breaking strength nor tibia breaking strength were affected by dietary treatment at 64 weeks ($P > 0.05$). These data indicate that aragonite may be used in place of limestone with positive improvements in egg quality until 52 weeks of age. Although egg production was not affected by the source of dietary calcium, the positive improvements in egg quality were less apparent at 64 weeks of age.

Key Words: limestone, dietary calcium, breaking strength, tibia, eggshell

P347 Effect of source and level of supplementary dietary zinc on growth performance and in vitro immune responses in broiler chickens Mohammad Pilevar*, Oluyinka Olukosi *Department of Poultry Science, University of Georgia*

A 21-day experiment was done to study the effects and modes of action of different dietary levels and sources of zinc (Zn) using growth performance and immune response in broilers chickens as response criterias. A total of 378 Cobb 500 (zero-day-old) were assigned to 9 treatments in a

randomized complete block design. Each treatment had 7 replicates, with 6 chicks per replicate cage. The diets were corn-soybean meal based and supplemented with 500 FTU/kg phytase. Treatments were in a 4×2 factorial arrangement, plus basal diet (0 mg Zn/kg). The factors were four sources of Zn ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, hydroxychloride Zn, Zn-Glycinate, and Zn-Lysine-Glutamate) and two levels of Zn (25 mg/kg and 100 mg/kg). The birds and feed were weighed on days 0, 10 and 20 to measure weight gain and feed intake. On day 21, a methyl thiazolyl tetrazolium (MTT) assay was used to determine the peripheral blood mononuclear cells (PBMC) proliferation response. Seven chickens (1 per replicate) were randomly chosen from each treatment to collect heparinized blood from the wing vein. There were no treatment effects on any of the growth performance responses at any point during the experiment. In vitro PBMC exposed to lipopolysaccharide (LPS) challenge showed that diminished cell viability caused by the challenge factor was improved by source of Zn supplementation ($P < 0.05$) at day 21. Compared to the control diet, birds receiving Zn as Zn-lysine-glutamate had significantly ($P < 0.05$) improved immune response whereas the other sources were at similar level as the control. The proliferation of PBMC in response to Concanavalin A was not influenced either by sources or levels of Zn supplemented in broiler diets. In conclusion, broiler chickens maintained the same growth performance irrespective of the level or source of Zn in the current experiment implying that birds receiving phytase-supplemented diets can utilize lower than 100 ppm dietary Zn supplementation. In addition, response to LPS challenge indicated that using Zn-lysine-glutamate had some advantage although it is not clear whether this is due to the Zn itself or its interaction with the matrix in which it is presented.

Key Words: Zinc, inorganic, Organic, immune response, broilers

P348 Effect of copper sources on performance, bone characteristics and mineral apparent retention in broilers. Valmiro Lima Aragao Neto*, Maiana do Nascimento Rhumas, Geiza Jesus dos Santos, Tatiane Almeida Viana Lopes, Jeronimo Goncalves de Brito, Alexandre Moraes Pinheiro *Universidade Federal do Recôncavo da Bahia*

The poultry industry has used copper at levels above its requirement as a tool to improve broiler performance due to its antibacterial activity. However, high copper levels can result in negative interactions with enzymes and other minerals. A study was conducted to evaluate the effects of inorganic copper as copper sulfate at two levels, 15 and 125 mg/kg, and Organic Copper (complexed to hydroxy-2-methyl-4-thio butanoic) (HMTBA) at 50 mg/kg of feed in two nutrient levels of recommendations Rostagno *et al.* (2017) (R), and Cobb (2018) (C) in a 3×2 factorial arrangement of treatments with 8 replicates per treatment combination. A total of 1,200 one-day-old Cobb 500 male chicks were randomly distributed among 48 floor pens (25 chicks/pen) and raised up to 38 d. BW and feed intake were recorded to calculate BW gain and feed conversion ratio. At 18d, one chicken per pen was selected for tibia collection to analyze weight, ash content, and seedor index, calculated by the relationship between weight and length. At 22d, 14 chickens per treatment were divided in 7 cages for excreta collection and subsequently analyzes of Ca and P retention using indigestible marker methodology. Data were analyzed using a two-way ANOVA with copper sources and nutrient recommendations as main effects in a factorial design. Mean separation was performed with Tukey's at a 5% significance level. No significant interaction effects ($P > 0.05$) were observed on BW, feed intake, BW gain or feed conversion ratio nor seedor index, and apparent calcium retention. Inorganic copper at 125 mg/kg reduced ($P < 0.05$) ash content only in R nutritional levels. Copper sulfate at 125 mg/kg reduced the phosphorus retention in the C nutritional standard, but not in the R standard. Regardless the nutritional recommendations, organic copper had higher ($P = 0.003$) Calcium retention, tibia weight ($P < 0.001$) and Seedor Index ($P < 0.001$) than Copper sulfate 125 mg/kg. R had better ($P < 0.001$) BW, FI and FCR than C nutritional recommendations. In conclusion, high levels of copper sulfate may pro-

voke negative effects on bone characteristics, that can be minimized using HMTBA Copper, with similar performance results.

Key Words: Chickens, minerals, organic, supplementation

P349 Effect of coccidiosis vaccine challenge on health, growth performance and plasma vitamin absorption of broiler chickens fed optimum dietary vitamins Hong Liu¹, Jose-Otavio Sorbara², Jose-Maria Hernandez², Jingcheng Zhang¹ *DSM China Animal Nutrition Research Center Co., Ltd., ²DSM Nutritional Products*

In this study the effect of coccidiosis vaccine challenge on health, growth performance and plasma vitamin absorption of broiler chicks with optimum dietary levels of vitamins (OVNTM) were evaluated. A total of 840 fourteen-day-old male broiler Cobb 500 chicks were allocated to two treatments: control and challenged group. On day 14, birds in challenged group were given a 30-fold dose of coccidiosis vaccine while the others in the control group were given the same amount of sterile water. All birds were provided with the diet containing optimum dietary levels of vitamins (OVNTM). The trial lasted from 14 to 28 days with 12 replicate pens for each treatment and 35 chicks in each pen. Coccidiosis lesion scores and plasma vitamin absorptions were determined at 7-day post-challenge. Results showed that, coccidiosis vaccine challenge resulted in greater ($P < 0.05$) coccidiosis lesion scores and poorer ($P < 0.05$) growth performance and plasma absorptions of vitamin A, α -tocopherol and 25-OH-D₃. Birds fed OVN-diet in the control group performed superior to the Cobb 500 objectives, while the performance was similar to the Cobb 500 objectives when the birds were challenged with coccidiosis vaccine. There was a strong positive correlation ($R = 0.976$, $P < 0.001$) between i-check quick-test α -tocopherol and HPLC analytical α -tocopherol. The present results suggest that the use of OVNTM-diet may exhibit beneficial effect on growth performance.

Keywords: vitamins, health, growth performance, broiler

Key Words: vitamins, health, growth performance, broiler, coccidiosis

P350 Performance, intestinal morphology, and oxidative status in coccidiosis-challenged broilers fed different doses of Cu and Zn from potentiated zinc and copper (I) oxide Agathe Romeo¹, Naiana Einhardt Manzke¹, Somayeh Pouraghaali², Mahdi Zhandi², Mojtaba Zaghari² *¹Animine, ²University of Tehran*

Coccidiosis, caused by parasites of the genus *Eimeria*, is known to cause drastic reductions in performance and induce mortality, thereby affecting the overall health status of poultry. The objective of the study was to evaluate the effect of different doses of Cu and Zn from a potentiated zinc source and copper (I) oxide on the performance, gut morphology, and oxidative status of challenged broilers. A total of 288 one-day-old male chicks (Ross 308) were selected and randomly allocated to 18 groups ($2 \times 3 \times 3$) with 4 pens and 4 chicks in each pen. Three levels of zinc (0, 80, and 160 ppm) and three levels of copper (0, 15, and 150 ppm) were tested with or without challenge. Broilers were orally inoculated with sporulated oocysts of four *Eimeria* species (*Eimeria tenella*, *Eimeria maxima*, *Eimeria arceculina*, and *Eimeria necatrix*) on day 15 of the study. Growth performance, intestinal morphology, oxidative status, and oocyst excretion were evaluated at the end of the experiment (day 42). The coccidiosis challenge had a negative impact ($P < 0.01$) on weight gain and feed conversion ratio, along with reduced villi height and crypt depth compared to broilers not challenged. Increasing the dose of both copper (I) oxide and potentiated Zn increased ($P < 0.01$) crypt depth and villi height to crypt depth ratio (V/C ratio), with a significant interaction ($P < 0.01$) between Cu and Zn for villi height, crypt depth, and V/C ratio when the higher doses were used in challenge broilers. Oocysts per gram of feces decreased significantly ($P < 0.01$) with the increase of zinc and copper in the diets and there was a significant ($P < 0.01$) interaction between copper and zinc when the higher doses were applied. The oxidative status was not significantly affected by the treatments. In conclusion, increasing levels of Zn and Cu from potenti-

ated zinc and copper (I) oxide in the diet may mitigate the negative effect of coccidiosis challenge on broilers.

Key Words: Trace-minerals, Eimeria, Poultry

P351 Determination of the optimum standardized ileal digestible calcium to phosphorus ratio in broilers Carrie Walk^{*1}, Chongxiao (Sean) Chen², Amin Rahimi², Chelsea Phillips³, Thomas Frost³, Lisa Laparade³, Aaron Cowieson¹ ¹*DSM Nutritional Products*, ²*North Carolina State University*, ³*DSM Nutritional Products NA*

An experiment was conducted to estimate the optimum standardized ileal digestible (SID) calcium to available P (avP) ratio for broilers. Straight-run, Ross 308 broilers (n = 2,592) were allocated to one of 8 diets in floor pens. There were 27 birds/pen and 12 pens/diet. The diets were arranged as a 4 x 2 factorial, with 4 SID Ca:avP ratios (0.80, 1.10, 1.40 or 1.70) and two levels of avP (nutrient adequate or deficient by 0.13% avP). All diets contained 1,000 FYT/kg phytase. Data were subjected to an ANOVA and the model included ratio, avP and the interaction. The optimum dietary SID Ca:avP ratio was estimated using significant parameter estimates for body weight gain (BWG), tibia ash, and plasma Ca. From hatch to d12, birds fed the low or adequate avP had the highest (quadratic, $P < 0.05$) BWG at 0.80 or 1.10 SID Ca:avP ratio, respectively (ratio x avP, $P < 0.05$). Feed conversion ratio (FCR) from hatch to d12 and BWG or FCR from d13 to 21 was optimized (quadratic, $P < 0.05$) in birds fed 1.10, 1.10 or 1.40 SID Ca:avP, respectively. On d21, tibia ash weight (g) was greatest (quadratic, $P < 0.05$) in birds fed 1.10 SID Ca:avP. From d22 to 42, there was no effect of SID Ca:avP or interaction on BWG, FCR, or tibia ash. However, feeding low avP diets reduced ($P < 0.05$) BWG and tibia ash weight (g). On d12, plasma Ca was lowest in birds fed 1.10 SID Ca:avP and increased (quadratic, $P < 0.05$) as the dietary SID Ca:avP ratio increased, regardless of the dietary avP level. Whereas, feeding low avP ($P < 0.05$) or increasing the dietary SID Ca:avP ratio (linear, $P < 0.05$) decreased plasma P. On d21, plasma Ca was lowest in birds fed 0.80 SID Ca:avP and low avP (linear, $P < 0.05$) but not influenced by SID Ca:avP ratio with adequate avP (ratio x avP, $P < 0.05$). Feeding low avP ($P < 0.05$) or increasing the dietary SID Ca:avP ratio (linear, $P < 0.05$) decreased plasma P. From hatch to d21, the dietary SID Ca:avP ratio to optimize BWG was 1.02-1.04, FCR was 1.26-1.28, tibia ash weight was 1.08-1.32. Plasma Ca was lowest in birds fed diets containing a SID Ca:avP ratio of 0.80. The SID Ca:avP ratio to optimize performance and bone ash was estimated at $< 1.40:1$ from hatch to d21. The SID Ca:avP ratio did not influence growth performance or bone ash of broilers from d22 to 42.

Key Words: available phosphorus, broiler, digestible calcium, plasma minerals, calcium to phosphorus

P352 Calcium pidolate dietary supplementation, an interesting strategy in a context of strong reduction of calcium and phosphorus in broilers feeds. Clémence MARECAILLE^{*1}, Julián MELO², Xavier ROULLEAU¹, Christophe ALLENO³ ¹*DIETAXION*, ²*Universidad Nacional de Luján, Departamento de Tecnología*, ³*ZOOTESTS*

In animal nutrition, phosphorus (P) is an expensive mineral nutrient with limited global reserves. It also has a negative environmental impact when it is excreted in excess. Calcium (Ca) is known as negatively impacting the digestibility of phosphorus when it is in excess in a diet. Calcium pidolate is known as an ingredient promoting the absorption of calcium and phosphorus.

In this context, we studied the effect of 3 levels of strong reduction of total calcium and digestible phosphorus associated with a supplementation of calcium pidolate.

680 Ross 308 male broilers were divided into four groups: Control (C), C-10%, C-20% and C-30%.

The levels of total Ca were 0.90, 0.80, 0.75 and 0.70% respectively in the starter feeds, 0.75, 0.66, 0.60 and 0.60% in the grower feeds, 0.60,

0.50, 0.50, 0.50% in the finisher feeds. The levels of P_{dig} were 0.45, 0.405, 0.36 and 0.315% in the starter feeds, 0.38, 0.342, 0.304 and 0.266% in the grower feeds, 0.30, 0.27, 0.24 and 0.20% in the finisher feeds. The Ca/ P_{dig} ratios of these feeds ranged from 1.9:1 to 2.5:1.

Calcium pidolate (PIDOLin PCa®, DIETAXION) supplementation was added in all the treatments, at the same dose (300 ppm), to secure the diet in this context of strong reduction of these two minerals.

The parameters studied were feed consumption, growth, feed conversion ratio (FCR), European production index (EPI), bone quality and litter quality at 35 days.

Data were statistically analysed with the software XLStat. The tests used were ANOVA or Kruskal-Wallis depending on the parameters.

Up to -20% reduction in Ca and P_{dig} compared to the levels of the control diet (C-20%), there was no degradation of the growth, consumption, FCR, EPI or bone quality ($P > 0.05$). However, although not significant ($P > 0.05$), the strongest reduction of Ca and P_{dig} (C-30%) led to a numerical reduction of the consumption and growth.

Regarding litter quality, the reduction of calcium and phosphorus in the feed was associated with a numerical improvement of the scoring (2.7/5 for batch C, and 2.5/5 for the other treatments), although not significant ($P > 0.05$).

This study shows that in diets containing calcium pidolate, the reduction of Ca and P_{dig} levels by up to -20% does not negatively impact the performance and bone development of broilers.

Key Words: Calcium Pidolate, Broiler, Calcium, Phosphorus

P353 The Effects of Supplemental KeyShure® Copper on Tibial Dyschondroplasia in Meat Type Ducks Zachary Lowman^{*1}, Curtis Novak², Jimmy Jurgielewicz³ ¹*Balchem Corporation*, ²*Purina Animal Nutrition*, ³*Joe Jurgielewicz and Son LTD*

The first report of induction of tibial dyschondroplasia (TD) by copper (Cu) deficiency was noted in white leghorns in 1964. Since then, much research has focused on Cu absorption and its role in bone development. The Cu currently used in many commercial diets is from inorganic (IN) forms (sulfate/oxides) which is very susceptible to antagonists both in the diet and within the gastrointestinal tract. Copper binds tightly to metallothionein and when epithelial cells are sloughed it causes a decrease in absorption which can result in deficiencies, especially when feeding lower levels of IN Cu. Tibial dyschondroplasia is known to be associated with rapid growth, however altering trace mineral levels (Cu, Mn, Zn) in the diet have shown to significantly alleviate TD. The objective of this experiment was to determine the effects of feeding organic Cu (KeyShure® Cu) on TD in meat type ducks. For this field trial, 2,880 meat type Pekin ducks were randomly assigned to 3 different dietary treatments (24 pens/120 ducks per pen): TRT 1 - Control (10ppm IN Cu), TRT 2 (50ppm KeyShure® Cu+10ppm IN Cu), or TRT 3 (100ppm KeyShure® Cu+10 ppm IN Cu). All ducks were reared under standard commercial conditions. At 14 d, 2 ducks were randomly selected from each pen and TD lesions were scored using a published duck TD scoring rubric (score of 0-4). Mortality was recorded throughout the 42 d grow out. Ducks from the TRT 3 group had numerically lower ($p=0.318$) TD scores than the control group (1.38 vs 2.19), with TRT 2 being intermediate (1.69). Body weights at 14 d demonstrated a similar trend where the control group had a lower BW (1.35lbs) compared to that of TRT 1 or TRT 2 (1.38lbs and 1.42lbs, respectively). The Control group had higher total mortality percent (2.39%) than TRT 3 (2.26%). As evident in this trial, adding KeyShure® Cu to the diet of meat type ducks leads to numerically improved TD scores, BW and decreased mortality when reared in a commercial setting.

Key Words: Pekin Duck, KeyShure® Copper, Organic Trace Minerals

P354 Effect of Zn Methionine-Hydroxy-Analogue Chelate supplementation on performance and carcass quality of broiler chickens. Liris Kindlein¹, Raquel Araujo², Fernando Magalhães², Fabio Zotese², Thiago Lantmann¹ ¹Universidade Federal do Rio Grande do Sul, ²Novus do Brasil Com. e Imp. LTDA.

Fourteen broiler producers with a history of the high incidence of pododermatitis in a company in southern Brazil were selected for an evaluation of effect of MINTREX[®] Zn supplementation on performance and carcass quality of broiler chickens. The first cycle was used as a negative control (T1), and the broilers flocks were not supplemented with MINTREX[®] Zn. In the second cycle, broiler flocks from the same 14 producers received supplementation with 40 ppm of MINTREX[®] Zn over the top, being also considered 0.02% of hydroxy analog of methionine, 2-hydroxy-4-(methylthio)butanoate (HMTBA), in the feed formulation. Six flocks received supplementation only in the final feeding phase (T2), 5 in all feeding phases (T3), and 3 only in the starter phase (T4). To evaluate the zootechnical performance, body weight gain (BWG), feed conversion ratio (FCR), slaughter age were measured. The adjusted feed conversion ratio (FCRa) for 2.5 kg of weight was calculated: $FCRa = [2.5-BWG]/3.7 + FCR$. For carcass quality, 400 birds/flock were used to observe: Callus chest; Skin lesions with yellowish scabs; Poorly healed scratches with inflammation; Healed scratches; Skin breakage with no apparent damage; cellulite; arthritis; and pododermatitis. Data were subjected to analysis of variance (ANOVA-GLM) and means were compared by the Tukey test. For variables with heterogeneous variance, non-parametric analysis (Kruskal Wallis test) was used, such as qualitative parameters and lesions (%). Data were analyzed with average BWG as a covariate. Analyses were made considering the 4 treatments or only 2 treatments (T1 x MINTREX[®] Zn). MINTREX[®] Zn improved BWG over T1 in all treatments (3.000, 3.198, 3.153 vs 3.049 kg; T2, T3, T4 vs T1; Pvalue 0.0001). FCRa was better for T3 compared to T1 (1.477 vs 1.623; Pvalue 0.078). Regarding qualitative parameters and lesions (%), treatments with MINTREX[®] Zn had better results compared to T1: Inflammation scratch (0.18 vs 0.68; Pvalue 0.001); Healed scratch (7.00 vs 17.57; Pvalue 0.014); Skin breakage with no apparent damage (2.65 vs 4.07; Pvalue 0.014); Arthritis (2.78 vs 6.18; Pvalue 0.022); and footpad with no lesions (69.3 vs 75.4; Pvalue 0.052). In conclusion, MINTREX[®] Zn improved BWG and FCRa and overall carcass quality results across all treatments.

Key Words: Zinc Methionine, MINTREX[®] Zn, organic minerals, broiler carcass quality, pododermatitis

P355 Testing of a novel cell culture mineral antagonist model to evaluate zinc transport Shane Morgan, Zachary Lowman, Kenneth Sanderson, Kari Estes^{*} Balchem Corporation

Cell culture techniques have been used to evaluate mineral transport. However, due to the *in vitro* nature of the experiment, these types of models have been met with mixed reviews within the animal agriculture industry. The Caco-2 transport model is a standardized cell culture method used to determine zinc (Zn) absorption, however this method may be missing crucial antagonistic steps that would otherwise occur *in vivo*. Thus, the objective of this work was to modify the standard Caco-2 transport model to more closely mimic *in vivo* conditions of a broiler chicken with the addition of inositol-6-phosphate (IP6). Three Zn treatments were tested for absorption: A) Zn sulfate; B) Zn hydroxychloride; and C) standard media (control). Each Zn treatment was first exposed to a digestion step (using HCl) and then to one of three different zinc antagonist conditions prior to the standard Caco-2 transport model: TRT 1) Control- no antagonist present; TRT 2) base feed made of ground corn and soy; and TRT 3) 0.1 mg/mL IP6 (phytate). The different antagonist conditions were designed to simulate possible interactions with various feed components such as proteins or antinutrients. There were noted decreases of Zn absorption in TRT 2 (feed) vs TRT 1 (control) for the standard media ($p=0.293$) (0.5658 nmol Zn vs 1.2136 nmol Zn) and Zn hydroxychloride ($p=0.0253$) (3.3969 nmol Zn vs 4.1824 nmol Zn), but not for the Zn sulfate ($p=0.971$) (3.7183 nmol

Zn vs 3.7905 nmol Zn). The phytate model resulted in the greatest antagonistic environment with significant decreases ($p<0.03$) in Zn absorption of 35.9%, 25.1% and 19.8% for the control, Zn sulfate and Zn hydroxychloride, respectively, when compared to the control (TRT 1). Based on this data, the phytate antagonist model produces the most consistent antagonist response regardless of the source of inorganic Zn.

Key Words: Trace Minerals, Cell Culture, Antagonist, Zinc

P356 Using a cell culture antagonist model to evaluate zinc absorption from organic and inorganic trace minerals Shane Morgan, Zachary Lowman, Kenneth Sanderson, Kari Estes^{*} Balchem Corporation

Organic trace minerals (OTM) are often used in animal diets as a partial replacement for conventional inorganic trace mineral (ITM) sources due to protection from antagonists and thus increased bioavailability. Natural antagonists, such as phytate (IP6), have been used in *in vivo* trials to demonstrate the benefits of OTM on animal growth and productivity. However, *in vivo* research trials are costly and time consuming. The Caco-2 transport model is a standardized cell culture method used to determine Zn absorption in a more timely and cost effective manner, however this method may be missing crucial antagonistic steps that would otherwise occur *in vivo*. Thus, the objective of this work was to determine Zinc (Zn) absorption from both organic and inorganic sources in cell culture by modifying the standard Caco-2 transport model to more closely mimic *in vivo* conditions with the addition of IP6. Four Zn sources were tested for absorption: 1) Zn Sulfate (ITM), 2) Zn Hydroxychloride (ITM), 3) KeyShure Plus Zn[®] (OTM), and 4) standard media (control). Each Zn source was first exposed to a digestion step (using HCl) and then to one of three different zinc antagonist conditions prior to the standard Caco-2 transport model. The different antagonist conditions were designed to simulate possible interactions with various feed components such as proteins or antinutrients. Thus, these three conditions consisted of the following: Condition A) Std media, no antagonist present; Condition B) Broiler base feed made of ground corn and soy; and Condition C) 0.1 mg/mL inositol-6-phosphate (IP6). The antagonist conditions negatively affected absorption for all Zn treatments, however the OTM (KeyShure Plus[®]) demonstrated the highest absorption in all three conditions, and the IP6 treatment resulted in statistically significant ($p<0.0001$) decreases in Zn absorption for the two ITM treatments (Trt 1 and 2; 2.839 nmol Zn and 3.3525 nmol Zn, respectively) compared to the OTM (4.988 nmol Zn). Based on these data, KeyShure Plus[®] is more capable of withstanding the negative effects of antagonists present in corn/soy based diets compared to Zn sulfate and Zn Hydroxychloride sources.

Key Words: KeyShure Plus, Organic Trace Minerals, Cell Culture, Antagonist

P357 The effect of various levels of dacitic (rhyolitic) tuff breccia on the antioxidant system and nutrient digestibility of broiler chickens mildly challenged with Eimeria spp. Po-Yun Teng¹, Janghan Choi¹, Sudhir Yadav¹, Fernanda Lima Souza Castro², Jon Ferrel², Woo Kim¹ ¹University of Georgia, ²AZOMITE Mineral Products, Inc.

The aim was to investigate the effect of a dacitic tuff breccia (DTB) with a mild *Eimeria* spp. infection. A total of 600 1-d-old Cobb 500 males (44.81g; SD \pm 0.26g) were assigned to a randomized complete block design of 5 treatments, 10 replicates of 12 birds for 26d. Birds were housed in cages (0.09 m²/bird) with *ad libitum* water and feed (corn-soybean-meal diets; starter (0-14d), grower (14-26d)). Treatments comprised of unchallenged (UC) and challenged controls (CC), and 3 challenged groups containing 0.125, 0.25, or 0.50% DTB. On d14, CC and DTB birds were orally gavaged with a pool of sporulated oocysts (12,500 *E. maxima*, 12,500 *E. tenella*, and 62,500 *E. acervulina*), and UC received water (1 mL). At d20, ileal digesta of 5 birds/cage were collected and pooled every 2 replicate cages for dry matter (DM), crude protein (CP) digestibility, and ileal digestible energy (DE). Liver sample activity of glutathione peroxidase

(GPx) was measured at d20, and concentrations of reduced (GSH) and oxidized glutathione (GSSG) were evaluated at d20 and d26. Data were analyzed using GLIMMIX (SAS v9.4) and Tukey's HSD with significance at $P < 0.05$ and trend at $P < 0.1$. DM and DE (treatment $P < 0.001$; challenged linear $P > 0.129$) favored UC vs. challenged groups (DM: 73.64 vs. 54.38, 54.52, 52.44, 49.33; $P < 0.001$ and DE: 2998 vs. 2140, 2132, 2118, 1895; $P < 0.001$). CP (treatment $P < 0.001$; challenged quadratic $P = 0.097$) favored UC vs. challenged groups (79.13 vs. 46.51, 57.03, 52.49, 47.81; $P < 0.001$). At 20d, GPx (mmol/min/g) was lowest for UC (0.027 vs. 0.036, 0.042, 0.041, 0.037; $P < 0.001$; treatment $P < 0.001$; challenged quadratic $P = 0.052$) and GSH (mmole/g) was highest in UC (4.27 vs. 3.24, 2.83, 2.84, 2.83; treatment $P < 0.001$; challenged $P = 0.654$). At 26d GSH was intermediate for UC and highest for 0.125% DTB (3.78 vs. 3.29, 3.97, 3.15, 2.73; treatment $P = 0.005$; challenged $P = 0.01$). No d20 or d26 GSSG treatment differences were observed (treatment $p > 0.234$; challenged $p > 0.218$). In conclusion, the *Eimeria* spp. infection negatively impacted nutrient digestibility, whilst 0.125% DTB showed potential in improving CP digestibility and GPx activity whilst challenged treatments at d20. The data also suggests a recruitment of GPx and GSH system due to the *Eimeria* spp. infection.

Key Words: Azomite, Dacitic Tuff Breccia, Broiler, Coccidiosis

P358 Lower levels of supplemental organic trace minerals vs inorganic trace minerals increased antioxidant status and humoral immune response in slow growing birds Marquisha Paul^{*}, Rebecca Delles, Jacqueline Jacob, Michael Ford, Tuoying Ao, Anthony Pescatore, Ronan Power *Alltech-University of Kentucky Nutrition Research Alliance*

A study was conducted to evaluate the effects of trace mineral source and level on the performance, antioxidant status and humoral immune response of slow growing meat birds. An RCB design with 4 trt and 10 replicate groups/trt was used for this study. A total of 240, one day old, black sex-linked chicks were blocked by sex (120 female, 120 male) and randomly assigned to 4 corn-soy diets: 1) 100% inorganic trace minerals (ITM; inorganic Cu, Mn, Zn and Se supplemented at 15, 100, 100 and 0.3 mg/kg, respectively), 2) 67% organic trace minerals (OTM; Bioplex® Cu, Mn, Zn and Sel-Plex® supplemented at 67% level of diet 1), 3) 50% OTM (supplemented at 50% level of diet 1), 4) 33% OTM (supplemented at 33% level of diet 1). Black sex-linked birds were raised in cages (6 chicks/cage) with *ad libitum* access to water and feed for 56 days in an environmentally controlled room with 22L: 2D photo-stimulation. Sheep red blood cell (SRBC) or phosphate buffered saline (PBS) was venously administered to chicks at 21 and 35 days of age to evaluate primary and secondary humoral immune responses. Data were subjected to ANOVA with mean comparisons using the LSD test. Growth performance over the 56-day study period was the same for black sex-linked birds provided 100% ITM, 50% OTM, or 33% OTM. Serum antioxidant concentration at 28 days of age was reduced ($P < 0.01$) in birds challenged with SRBC vs PBS, however SRBC challenged birds provided 67% or 33% OTM exhibited higher ($P = 0.08$) serum antioxidant concentration than those provided 100% ITM. Serum immunoglobulin titers were not different ($P > 0.05$) among trts after the primary SRBC challenge. After the secondary SRBC challenge, birds provided 67% OTM had the highest ($P < 0.05$) total immunoglobulin (Ig) and IgM titers compared to the other trt groups, however there were no differences in serum immunoglobulin titers among the other trt groups. Results from this study showed that dietary organic trace minerals at lower levels than inorganic trace minerals improved the antioxidant status and humoral immune responses of slow growing birds.

Key Words: Organic minerals, Slow growing birds, Antioxidant, Immune response, growth performance

P359 Texture profile analysis of raw pet treats generated from broiler chicken wing tips Justin A Dunavant^{*GS1}, Jorge R Romero¹, John W Rogers¹, Marc R Presume¹, Said J Herrera¹, Diego E Ventura¹, Josh S Renew¹, Jorge L Sandoval¹, Jorge E Banegas¹, Gerardo A Abascal-Ponciano¹, Christopher I Almendares¹, Luis J Guzman¹, Tristan M Reyes², Madison P Wagoner², Robert P Mason³, Eric K Altom³, Jason T Sawyer², Jessica D Starkey¹, Charles W Starkey¹ ¹Auburn University Department of Poultry Science ²Auburn University Department of Animal Science ³Balchem Animal Nutrition and Health, Balchem Corp.

Broiler chicken wing tips (WT) are a low value co-product generated from broiler processing. When forming agents are used in ground meat products, a more structurally stable product is created. The objective of the experiment was to assess the effect of increasing inclusion of a forming agent (ALGIN) containing sodium alginate (SA) and encapsulated calcium lactate (ECL) on texture profile analysis (TPA) of pet treats generated from previously frozen WT. Chilled WT were ground with a 4.8-mm grinder plate and mixed with 1 of 4 different concentrations ALGIN: 0x (no ALGIN inclusion), 0.5x (0.425% ECL + 0.5% SA), 1x (0.85% ECL + 1% SA), and 2x (2% ECL + 1.7% SA). After mixing, each treatment was extruded into 63.5-mm diameter casings, stored overnight at 4 °C, and frozen at -20 °C for 3 h to facilitate slicing into 5-mm-thick discs for analysis. After slicing, treats were held at 4°C until all samples could be analyzed (< 48 h). Using a texture analyzer (Stable Micro Systems, TA-HDplusC model), treat TPA was measured using two-cycle compression with a TA-40-cylinder probe to obtain values for 6 attributes: hardness (maximum force required to deform), cohesiveness (structural integrity under compression), adhesiveness (adhesion of sample to the probe), chewiness (energy required to masticate), resilience (ability of sample to regain original form), and springiness (proportion of height that the sample recovers between mastication). Data were analyzed as a 1-way ANOVA using the GLIMMIX procedure of SAS and means were separated at $P \leq 0.05$ with the PDIFF option. No differences in treat resilience ($P \leq 0.1899$) or cohesiveness ($P \leq 0.3215$) were observed. Treats containing 0x and 0.5x ALGIN were similar in hardness and chewiness, while hardness and chewiness were greater in treats with 1x ALGIN and greater again in treats with 2x ALGIN ($P \leq 0.0001$). The greatest adhesiveness was observed in treats containing 1x and 2x ALGIN, while those with 0x were the least adhesive and 0.5x-ALGIN-containing treats were intermediate ($P \leq 0.0001$). Treats containing 2x ALGIN had greater springiness than those with 0.5x and 0x but were not different from those with 1.0x ALGIN ($P \leq 0.0001$). Inclusion of a forming agent altered the textural properties of raw pet treats.

Key Words: TPA, wing tip, sodium alginate, pet treats, texture analysis

P360 Sample size, outlier removal, and tibial bone metric selection for best representation of 56 d Ross x Ross 708 males fed diets varying in available phosphorus Samantha Plocher¹, Dalton Dennehy¹, N. Tillman², Donna Morgan¹, Kelley Wamsley¹ ¹Department of Poultry Science, Mississippi State University ²Nutritional Statistics

Previous research examining sample size (SS) required for tibial bone status in laying hens determined SS needed was dependent upon bone status method utilized; though, this has not been fully discussed in broilers. The objective of this study was to compare SS, outlier removal (OR), and metric to best represent bone mineralization use tibia ash (TA) data. The data used were from a trial using Ross x Ross 708 males fed one of 4 diets varying in available P. Chicks were randomly placed (14 birds/pen) into one of 48 floor pens (experimental units). Birds were reared for 56 d then individually weighed; and each bird's right tibia was excised, dried, defatted and ashed. Four measurements were collected and calculated: BW, TA (g/bird), % TA ((g of TA/dried tibia weight)*100); TATW, and % TA relative to BW ((g of ash/g of BW)*100; TABW). Data were analyzed according to a One-Way ANOVA. Fisher's LSD was utilized after ANOVA with $P \leq 0.05$. Four SS procedures (SSP) were used to collect the data from BW and corresponding TA measurements; for each analysis, the average per pen was used. The SSP consisted of the following: SSP1) 1 bird +/-

100 g of average BW/pen (total# birds measured (TBM) =48, n/pen=1, n/trt=12); SSP2) 5 random birds/pen (TBM=240, n/pen=5, n/trt=12); SSP3) 1 randomly selected bird/pen (TBM=48, n/pen=1, n/trt=12); and SSP4) all birds/pen (TBM=383, n/pen=11-14, n/trt=12). For each SSP, outliers were eliminated using Cook's distance $>4/n$ and identified as SSPOR1-4. Outlier elimination had the following results: SSPOR1) TBM=45-46, n/pen=0-1, n/trt=10-12); SSPOR2) TBM=228, n/pen=3-5, n/trt=10-12); SSPOR3) TBM=46, n/pen=0-1, n/trt=8-12); and SSPOR4) all birds/pen (TBM=366, n/pen=10-14, n/trt=12). As expected, eliminating outliers from each SSP decreased SEM for each variable. In general, SSP demonstrated similar means separation; though sampling SSP4 was most sensitive and had the lowest SEM (especially with SSPOR4). Noteworthy, TABW analyses were not as consistent, whereas utilizing SSP1 and SSPOR1 revealed differences in significance ($P=0.2895$ and 0.0291 , respectively). Although d 56 TA metrics are less sensitive than younger chicks, differences noted within the current study demonstrate different results could be obtained depending upon SSP and outlier elimination.

Key Words: sample size, tibia, phosphorus, broiler

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