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Abstracts of the Poultry Science Association Annual Meeting

SYMPOSIA AND ORAL SESSIONS

Monday, July 23, 2018

Tuesday, July 24, 2018

Wednesday, July 25, 2018

Thursday, July 26, 2018

Posters

Symposia

Student Competition: Immunology, Health, and Disease I

1 High-throughput screening for natural host defense peptide-inducing compounds as novel alternatives to antibiotics. W. Lyu*, Z. Deng, R. Matts, and G. Zhang, *Oklahoma State University, Stillwater, OK.*

A rise in antimicrobial resistance demands novel alternatives to antimicrobials for disease control and prevention. As an important component of innate immunity, host defense peptides (HDPs) are capable of killing a broad spectrum of pathogens and modulating a range of host immune responses. Enhancing the synthesis of endogenous HDPs has emerged as a novel host-directed antimicrobial therapeutic strategy. To facilitate the identification of natural products with a strong capacity to induce HDP synthesis, a stable macrophage cell line expressing a luciferase reporter gene driven by a 2-Kb avian β-defensin 9 (AvBD9) gene promoter was constructed through lentiviral transduction and puromycin selection. A high throughput screening assay was subsequently developed using the stable reporter cell line to screen a library of 584 natural products. A total of 21 compounds with a minimum Z-score of 2.0 were identified. Secondary screening in chicken HTC macrophages and jejunal explants further validated most compounds with a potent HDP-inducing activity in a dose-dependent manner. A follow-up oral administration of a lead natural compound, wortmannin, confirmed its capacity to enhance the AvBD9 gene expression in the duodenum of chickens. Besides AvBD9, most other chicken HDP genes were also induced by wortmannin. Additionally, butyrate was also found to synergize with wortmannin and several other newly-identified compounds in AvBD9 induction in HTC cells. Therefore, these natural HDP-inducing products may have the potential to be developed individually or in combinations as novel antibiotic alternatives for disease control and prevention in poultry and possibly other animal species including humans.

Key Words: host defense peptides, antimicrobial peptides, high-throughput screening, wortmannin, antibiotic alternatives

2 Effect of dietary spray-dried plasma on immunocompetence in broiler chicks. C. Blue*[1], Y. Jababu[1], L. Young[1], R. Ali[2], M. Koci[2], and Y. Fasina[1], *[1]North Carolina A&T State University, Greensboro, NC, [2]North Carolina State University, Raleigh, NC.*

Due to consumer pressure, there is a gradual reduction in the use of antibiotic growth promoters (AGPs) broiler chicken production. Spray-dried plasma (SDP) is a potential alternative to AGPs because they contain various functional proteins such as albumin, immunoglobulins, growth factors, and biologically active peptides that are capable of stimulating the immune system. An experiment was conducted to evaluate the effect of porcine SDP supplementation on immunocompetence in broiler chicks. Day-old (240) Ross 708 male chicks were obtained from a commercial hatchery, weighed, and randomly assigned to 6 dietary treatments. Treatment 1 (CX) consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without SDP. Treatment 2 (MX) consisted of chicks given unmedicated corn-SBM basal into which Bacitracin methylene disalicylate (BMD) was added at 0.055g/kg diet. Treatments 3 (SP1), 4 (SP2), 5 (SP3), and 6 (SP4) consisted of chicks given unmedicated corn-SBM basal into SDP was added at 10, 20, 30, and 40 g/kg diet, respectively. Each treatment consisted of 4 replicate pens, with each pen housing 10 chicks. Chick growth performance (body weight and feed conversion ratio (FCR)) was monitored throughout the 28-d experiment. On d 14 and 21, blood was collected and subjected to differential leukocyte count (DLC) analysis and the estimation of serum IgG concentration by enzyme-linked immunosorbent assay. Lymphoproliferative response to kidney bean lectin *(*PHA-P) as an indicator of T-cell-induced delayed type hypersensitivity (DTH) was also evaluated from d 26 to d 27 of experiment. Data collected was subjected to one-way ANOVA, and means separation was done using Duncan's multiple range test. Although differences were not observed in average body weight ($P > 0.05$), FCR of chicks in SP3 (1.25) and SP4 (1.24) was superior ($P < 0.05$) to CX (1.31), and similar to MX (1.29). Circulating IgG concentration was higher for SP1, SP2, and SP3 treatments (2955–2975 ng/mL; $P < 0.05$), compared with MX (2933 ng/mL). DTH response of MX treatment was higher ($P < 0.05$) was higher than that of CX, while those of SDP-supplemented treatments were in between. Specifically, SP1 and SP3 had similar DTH response to MX ($P < 0.05$). It was concluded that incorporation of SDP at a minimum of 30g/kg diet could enhance immunocompetence in broiler chicks.

Key Words: spray-dried plasma, immunocompetence, delayed type hypersensitivity, growth performance

3 In ovo application of epidermal growth factor in late embryonic stage on growth performance, gut development, and nutrient utilization in broiler chickens. E. Kim*, N. Akhtar, J. Li, and E. Kiarie, *University of Guelph, Guelph, ON, Canada.*

We previously reported that epidermal growth factor (EGF), a protein known for its mitogenic and antiapoptotic effects improved broiler growth performance and increased gene expression for digestive enzymes, nutrient transporters, cytokines, proliferating cell nuclear antigen, and tight junction proteins upon challenge with *Eimeria*. The aim of this study was to investigate the efficacy of in ovo application of EGF in late embryonic stage on growth performance, nutrient retention, and gastrointestinal (GIT) development. A total of 600 Ross 708 fertile eggs were allocated to 5 treatments (120 eggs/treatment). The treatments included: 1) intact (no punching and no injection), 2) punched and no injection, 3) control (injected with fermentation supernatant without EGF), 4) 80 µg of supernatant containing EGF/kg of egg, and 5) 160 µg of supernatant containing EGF/kg of egg). The eggs were incubated and candled for live embryos on d 19. The viable eggs were subsequently injected and transferred to the hatcher. Upon hatching, 90 chicks of each treatment were placed in cages (15 birds/cage) and the remaining chicks were necropsied for baseline GIT weights (gizzard, small intestine, and ceca) and jejunal histomorphology. Birds had free access to water and standard antibiotic-free corn-soybean diet containing TiO_2 as indigestible marker for 21 d post-hatch. Feed intake and BW was monitored weekly and excreta samples were taken on d 3–5 and d 17–19 for AR of DM. On d 7, 14, and 21, 5 birds per cage were necropsied for GIT weights and jejunal histomorphology. All parameters were subjected to a one-way ANOVA with treatment as a fixed factor and contrast statements to look at the effect of injection, punching, and EGF. There was no effect of punching, injection, or EGF on ($P > 0.05$) on any response criteria. The final BW were 845.5, 855.9, 891.4, 902.9, and 898.0 g respectively and corresponding FCR were 1.34, 1.40, 1.49, 1.34, 1.34, respectively. The AR of DM for d 3–5 were 70.29%, 70.71%, 70.68%, 70.1%, 70.4% respectively and the corresponding values for d 17–19 were 74.19%, 74.9%, 74.39%, 74.96%, 74.16%. These results demonstrated that in ovo injection of EGF had negligible effects on growth performance, nutrient retention, and indices of gut development. The lack of EGF effect was surprising, as EGF binds to the EGF receptor to activate signal transduction pathways involved in

regulating cellular survival, growth, proliferation, and differentiation. However, it is unclear whether chick embryos possess the receptors or if the injected EGF was degraded by proteases in the amniotic fluid before delivery into the developing gut.

Key Words: broiler, in ovo, epidermal growth factor, gut health and function

4 Identification of cells expressing avian beta defensin mRNA in the chicken yolk sac and small intestine. H. Zhang* and E. Wong, *Virginia Tech, Blacksburg, VA.*

During the embryonic and early posthatch periods, the chicken immune system has not fully matured, yet chicks are susceptible to infection from pathogens. The chicken yolk sac and small intestine play a similar role in nutrient absorption and serve as a physical barrier to invasion by potential pathogens present in the yolk or intestinal lumen, respectively. Avian β defensins (AvBDs) are host defense peptides that are part of the chicken innate immune system. The objectives of this study were to profile the expression of AvBD mRNA using quantitative PCR (qPCR) and to identify by in situ hybridization cells that express AvBD mRNA in the yolk sac and small intestine during the embryonic and early posthatch periods. qPCR data were analyzed by ANOVA and further separated by Tukey's test with the significance level at 0.05. The transcriptional profile of AvBD mRNA in the yolk sac showed that AvBD1, 2, 7, and 10 were highly and differentially expressed from embryonic d 7 (e7) to e19. In situ hybridization and Giemsa staining revealed that AvBD1, 2, and 7 mRNA were mainly expressed in activated acidophilic granulocytes in the yolk sac, which were presumably chicken heterophils. AvBD10 mRNA was predominantly expressed in yolk sac endodermal epithelial cells. The expression profile of AvBD10 mRNA showed an increase from e7 to e11 and then a decrease to low levels at e15 and e19, when analyzed by both the in situ hybridization and qPCR assays. In the small intestine, AvBD10 mRNA was expressed by intestinal epithelial cells along the intestinal villus at e19 and day of hatch. However, by d 2, only a few cells above the intestinal crypts expressed AvBD10 mRNA. In summary, AvBD10 was expressed in the epithelial cells of the yolk sac and intestinal villi, while AvBD1, 2, and 7 were expressed in acidophilic granulocytes, suggesting that these AvBD play an important role in chicken innate immunity.

Key Words: avian β defensins (AvBD), yolk sac, small intestine

5 Immunoassay and immunoaffinity purification of chicken high mobility group box 1 (chHMGB1), a potential new inflammation marker. N. A. Ali*, C. Vuong, W.-K. Chou, and L. Berghman, *Texas A&M University, College Station, College Station, TX.*

High mobility group box 1 (HMGB1) was originally described as a highly conserved, 215 amino acid chromosomal protein that served as a DNA chaperone. However, more recently HMGB1 was revealed to also play an important role as a potent pro-inflammatory immune modulator. When released into the circulation by necrotic cells, HMGB1 becomes as a prototypical danger-associated molecular pattern (DAMP), although it can also be secreted by cells of the monocyte lineage under physiological conditions. As such, HMGB1 may serve as a biomarker for inflammation in various poultry diseases, such as coccidiosis and necrotic enteritis. Therefore, mouse monoclonal antibodies were generated against the amino-terminus (1–16) and the cytokine-inducing region (90–111) of chHMGB1. In addition, polyclonal rabbit sera were developed against chHMGB1 (156–177), the RAGE-binding region of chHMGB1. Different sandwich ELISA formats based on all possible antibody capture and detection antibody pairs were tested with a nuclear chromatin extract as the source of chHMGB1. The configuration featuring rabbit anti-chHMGB1 (156–177) as the capture antibody and either mouse anti-chHMGB1 (1–16) or mouse anti-chHMGB1 (90–111) produced by far the best signal to noise ratio. Both mAbs were successfully used for the immunoaffinity purification of chHMGB1 from a nuclear chromatin extract using mAb-functionalized magnetic beads, and the resulting chHMGB1 preparation will be used to standardize the above sandwich ELISA. Immunoblotting analysis of both affinity-purified chHMGB1 preparations using rabbit anti-chHMGB1 (156–177) for immunodetection revealed identical bands with an MW of approximately 65 kDa. These results suggest that chHMGB1 dimerized upon purification, similar to what has been described for mammalian HMGB1. Future research will focus on validating this ELISA for the measurement of chHMGB1 in chicken serum samples under physiological and pathological conditions.

Key Words: chicken, HMGB1, sandwich ELISA, monoclonal antibody, polyclonal antibody

6 Immunofluorescent characterization of professional antigen-presenting cells in chicken Peyer's patches using monoclonal antibodies against CD205 and CD40. W. Qu*, C. Vuong, C. Martin, M. Alabdali, N. A. Ali, J. Skrobarczyk, and L. Berghman, *Texas A&M University, College Station, TX.*

In the chicken, Peyer's patches (PP) represent a crucial gut-associated lymphoid tissue (GALT) responsible for antigen sampling and activation of T-cells and B-cells. This involves antigen presentation in the context of major histocompatibility complex class II (MHC-II) by professional antigen-presenting cells (APC).This study aims at elucidating the microanatomical organization of the APCs in the PP to better understand their role in initiating the response to orally administered vaccines. PP can be most readily identified in young birds (3–12 weeks of age) as an ovoid white patch about 1-cm in length on the antimesenteric side of the mucosa in the distal ileum between the ceca and cephalic to the cecal tonsils. The hallmarks that make the PP different from the adjacent intestinal tissue include thickened villi, heavy lymphocyte filtration and isolated follicles deeply embedded in the muscularis mucosa/submucosa of the intestine. For this study, PP and adjacent tissue were rolled up as a Swiss roll, snap frozen in liquid nitrogen (vapor phase), cryosectioned at 5μm and 10μm and fixed by cold acetone and 80% methanol before immunofluorescent visualization of CD205 (DCs), CD40 and/or MHC-II (APCs), IgM (B-cells) and CD3 (T-cells). In the center of the PP follicle, CD40 and surface IgM were abundantly expressed, while expression of MHC-II and CD205 was relatively scarce. $CD3^+$ cells were predominantly distributed in the peripheral zone of PP follicle and localized intraepithelially and in lamina propria of the adjacent villi. $MHC\text{-}II^+$ APCs were packed subepithelially throughout lamina propria and some were penetrating the follicle-associated epithelium (FAE) toward the lumen. $CD205^+$ DCs appeared as single cells near the crypts and were occasionally found inside the follicles. $CD40^+$ APCs were clustered both inside and outside the follicles. These results show that, much like in mammalian PP, naïve B-cells are the major cell type occupying the follicles of chicken PP, while T-cells can be found in the interfollicular areas.

Key Words: chicken Peyer's patches, dendritic cells, antigen-presenting cells, CD205, CD40

7 Oxidation of energy substrates by enterocytes of 0- to 42-day-old chickens. W. He*, K. Furukawa, H. Leyva-Jimenez, C. Bailey, and G. Wu, *Texas A&M University, College Station, TX.*

Glutamate, glutamine, and aspartate are known to be major energy substrates for mammalian enterocytes, but little is known about metabolic fuels in avian enterocytes. In the present study, enterocytes isolated from 0, 7, 21, and 42-d-old broiler chickens were used to determine the rates of oxidation of amino acids, fatty acids and glucose. Enterocytes were incubated at 40°C for 30 min in Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 5 mM D-glucose and one of the following: 0.5, 2 and 5 mM L-[U-^{14}C]glutamate; 0.5, 2 and 5 mM L-[U-^{14}C]glutamine; 0.5, 2 and 5 mM L-[U-^{14}C]aspartate; 0.5, 2 and 5 mM L-[U-^{14}C]alanine; 0.5 and 2 mM L-[U-^{14}C]palmitate; 0.5, 2 and 5 mM [U-^{14}C]propionate; 0.5, 2 and 5 mM [1-^{14}C]butyrate; or D-[U-^{14}C]glucose. At the end of the incubation, $^{14}CO_2$ produced from each ^{14}C-labeled substrate was collected into Soluene^{TM} for counting. Data were statistically analyzed by one-way or tw-way ANOVA. Rates of oxidation of each substrate by enterocytes from all age groups of chickens increased progressively with increasing extracellular concentrations. The rates of oxidation of glutamate by enterocytes from 0- to 42-d-old chickens were greater than those for the same concentrations of other substrates, and the same results were obtained for the rates of oxygen consumption. Compared with 0-d-old chickens, the rates of oxidation of glutamate, aspartate, glutamine, and glucose by enterocytes from 42-d-old chickens decreased by 80%, 60%, 35%, and 67%, respectively, but the rates of oxidation of alanine increased by 3-fold. Oxidation of propionate, butyrate and palmitate was limited in enterocytes from all age groups of chickens. Based on metabolic pathways, the oxidation of glutamate produced more ATP than any other substrates during the postnatal growth of chickens. Collectively, our results indicate that glutamate is the major metabolic fuel in enterocytes of 0- to 42-d-old chickens. Because of limited uptake of arterial glutamate by enterocytes, dietary (enteral) glutamate is essential to maintain the integrity and function of the chicken small intestine.

Key Words: energy substrates, small intestine, oxidation, glutamate, chickens

8 A meta-analysis of [^{13}C] bicarbonate, intravenous and intragastric [1-^{13}C] methionine infusions, and indirect calorimetry for measurement of CO_2 production in broilers. J. Weil*[1], P. Maharjan[1], K. Hilton[1], A. Beitia[1], G. Mullenix[1], M. Schlumbohm[1], J. England[1], V. Naranjo[2], and C. Coon[1], *[1]University of Arkansas, Fayetteville, AR, [2]Evonik Nutrition & Care, Hanau, Germany.*

To understand the degradation of amino acids within the broiler, stable isotopes and indirect calorimetry are used in combination to determine pre-systemic and systemic metabolism as well as whole body oxidation through decarboxylation. A total of 1000 Cobb 500 chicks were divided into 20 pens (50 chicks/pen) to determine the rate of CO_2 produced (VCO_2) through stable isotopes and indirect calorimetry. Broilers were fed a common starter diet (0–10d) and grower diet (10–21d) with different ratios of methionine and cysteine, ranging from deficient to excess dTSAA and dMet. Once the birds were 21d old, birds were selected for either control, metabolic chambers, or intravenous [1-^{13}C] sodium bicarbonate infusion. Additionally, birds were selected for intravenous (IV) or intragastric (IG) infusion of [1-^{13}C] Met. Birds selected for bicarbonate infusion were sampled at one of 5 time intervals (2, 10, 30, 45, 60 min post-infusion) while those infused with [1-^{13}C] Met were sampled at one of 11 time intervals (5, 15, 30, 45, 60, 120, 180, 240, 300, 360, 420 post-infusion). Ten non-infused birds served as controls for the labeled $^{13}CO_2$. Blood from jugular vein was collected in evacuated tubes and $^{13}CO_2$ enrichment was determined by isotope ratio mass spectrometry (IRMS). Results from the IRMS were converted to atom percent excess (APE) and analyzed with pharmacokinetic modeling of JMP Pro 13.1. Comparison between methods was analyzed using ANOVA, with means separated using Tukey HSD (JMP Pro 13.1). Birds selected for indirect calorimetry were placed in chambers for 48 h: 24 h for adaptation and 24 h for data collection. CO_2 production calculated through the chamber readings allowed for the calculation of a 13C recovery factor. Through the metabolic chambers, the average VCO_2 produced by whole body oxidation was 336.37 mmol/min/kg BW. Results from IV infusion of [1-^{13}C] sodium bicarbonate revealed CO_2 peaked by 10 min and was expired by 60 min. VCO_2 was determined to be 338.13 mmol/min/kg BW through this method. Bicarbonate infusion had an APE of 0.035 ($P < 0.0001$). Examination of IV and IG methods revealed IV infusion had a higher APE (0.004 vs 0.003, respectively) ($P = 0.0065$) compared with IG infusion, which suggests parenteral uptake allows more efficient delivery than enteral. Additionally, recovery of $^{13}CO_2$was 12% higher in IV infusions compared with IG infusions. Each of the 3 methods, regardless of delivery, prove useful in determining energy expenditure in broilers, considering compartmental and whole body oxidation is necessary for understanding metabolism.

Key Words: broiler, bicarbonate, indirect calorimetry, stable isotopes, CO_2

9 Developmental changes in the activities of enzymes for polyamine synthesis in chickens. K. Furukawa*[1], W. He[1], H. Leyva-Jimenez[1], C. Bailey[1], F. Bazer[1], M. Toyomizu[2], and G. Wu[1], *[1]Texas A&M University, College Station, TX, [2]Tohoku University, Sendai, Japan.*

Polyamines (putrescine, spermidine and spermine) are essential to DNA and protein syntheses and, therefore, the rapid growth and development of chickens. However, metabolic pathways for polyamine synthesis in avian tissues are largely unknown. In mammals, polyamines are produced primarily from arginine-, proline-, and glutamine-derived ornithine in a cell-specific manner and, to a lesser extent, arginine-derived agmatine in certain tissues (e.g., ovine conceptus). However, in chickens, expression of arginase is relatively low in all tissues, pyrroline-5-carboxylate synthase is absent from the intestine, and no data are available regarding proline oxidase (POX) in any tissues. Therefore, this study was conducted to determine developmental changes in: (1) the activities of enzymes (measured at 40°C under optimal conditions) for polyamine synthesis; and (2) concentrations of polyamines in chicken tissues. Kidney, jejunum (without luminal contents), leg muscle, liver, pancreas and plasma were collected from 0-, 7-, 21- and 42-d-old broiler chickens. Polyamines and enzymatic products were determined using our established HPLC and UV/VIS spectrophotometric methods. Data were statistically analyzed by one-way ANOVA. Results indicate that arginase and POX activities were present only in the mitochondrial fraction of the kidney in 0- to 42-d-old chickens. Renal POX activity was greater ($P < 0.01$) on Day 7 compared with Day 0, but no change in renal arginase activity was detected during this period ($P > 0.05$). Arginine decarboxylase and agmatinase were not detected in any tissues examined. Spermidine was the most abundant polyamine in all tissues and plasma of chickens. Interestingly, the concentrations of putrescine,

spermidine and spermine in the plasma of chickens were about 10-, 100-, and 10-fold greater ($P < 0.01$), respectively, than those in mammals. Consistent with enzymatic activities, concentrations of all polyamines in kidneys and plasma, and of putrescine in liver, were greater ($P < 0.01$) on Day 7 compared with Day 0 and, thereafter, values decreased ($P < 0.01$) on Days 21 and 42. Collectively, our results indicate that polyamines are synthesized from both arginine via arginase and proline via POX in the kidneys of chickens. We suggest that polyamines released from the kidneys are the major source of polyamines for extra-renal tissues.

Key Words: polyamines, arginine, proline, chicken, development

10 An in vivo technique for measuring the digestion rate of dietary proteins fed to poultry. D. D. S. L. Bryan*, D. Abbott, and H. Classen, *University of Saskatchewan, Saskatoon, SK, Canada.*

The rate and extent of protein digestion are relevant to broiler performance and health, but information is lacking on the rate of digestion for common high protein feed ingredients. An in vivo technique is proposed for measuring the digestion kinetics of protein sources fed to poultry. The method is a modification of the standardized ileal digestible assay. Using a completely randomized design, 360 male broilers at 14 d of age were assigned to 60 battery cages and fed semi-purified diets composed of wheat starch (N-free) or wheat starch with either blood meal (BM), canola meal (CM), corn distillers dried grains with solubles (CDDGS), corn gluten meal (CGM), feather meal (FEM), fish meal (FM), meat and bone meal (MBM), porcine meal (PM) or soybean meal (SBM). Feed intake (FI) was measured for 24 h before digesta sampling on d 21. Amino acid (AA) digestibility was determined for the proximal and distal sections of jejunum and ileum. The FI and indigestible marker of the diet were used to calculate the mean retention time (MRT) of digesta in each section of the small intestine. The MRT and AA digestibility along the small intestine were modeled to the first order kinetic curve using the PROC NLIN procedure of SAS 9.4 and the resulting rate constant (kd) data were analyzed as a one way ANOVA. Differences were considered significant when $P \leq 0.05$. Protein source affected the rate of AA digestion with average AA kd values for meals ranging from 0.016 to 1.841. The results demonstrated differences in AA kd among and within protein sources. For example, FM had the highest digestion rate for most of the AA evaluated among the protein sources, while CDDGS had the lowest. Methionine had the lowest kd of the AA in CDDGS, however, methionine along with lysine had the highest kd compared with the other AA in FM. In conclusion, dietary protein source affects the digestion kinetics of AA along the small intestine of broilers. This parameter may influence how AA from various protein sources are utilized for muscle deposition after they are digested and absorbed.

Key Words: digestion rate, soybean meal, corn gluten meal, corn distiller dry grain with solubles, amino acids

11 Protein turnover and performance parameters for modern commercial broiler strains fed varying levels of dietary amino acids and metabolizable energy. G. Mullenix*[1], J. England[1], K. Hilton[1], M. Schlumbohm[1], J. Caldas[2], A. Kalinowski[3], V. Naranjo[4], and C. Coon[1], *[1]University of Arkansas, Fayetteville, AR, [2]Cobb-Vantress, Siloam Springs, AR, [3]Evonik, Essen, Germany, [4]Evonik Nutrition & Care, Hanau, Germany.*

The objective of this study was to see how modern commercial broiler strains synthesize and degrade protein when fed varying dietary amino acid and metabolizable energy levels. Protein turnover was determined at 22d, 35d and 42d through intravenous flooding-dose of 15N-phenylalanine by fractional synthesis rate (FSR) and fractional breakdown rate (FBR). Delta values or difference between FSR and FBR were calculated for the experimental trial periods. Body weight (BW), average daily gain (ADG), feed conversion ratio (FCR), and protein turnover were evaluated. Grams of digestible Lysine per Mcal was used to compare the nutrient density across both trials. Two trials with 2 thousand 25 Cobb MX × Cobb 500 and Ross YP × Ross 308 were placed in 90 pens (n = 45 birds/pen) for the study. Commercial starter and grower feed were fed 1–10d and 11–22d, respectively. Five experimental finisher diets were fed 22–42d in pellet form (9 reps per strain/diet) in each trial. The varying amino acid finisher diets were isoenergetic (3125 kcal/kg) and formulated to the ideal amino acid recommendation of AMINOChick®2: 0.80%-, 0.90%-, 1.00%-, 1.10%- and 1.20% dLys, respectively. Other first limiting amino acids were held at a constant ratio to dLys level: Met+Cys, 0.76; Thr: 0.65; Val: 0.80; Ile: 0.71; Arg: 1.05, and Trp: 0.16. The finisher metabolizable test diets were iso-nitrogenous and formulated to different AMEn levels: 2800, 2925, 3050, 3175 and 3300 kcal/kg. All diets were formulated to AMINOChick®2 recommendations, with dLys set at 1.00% and other amino acids set as a ratio to dLys. There were significant differences from strain ($P = 0.0479$) ($P = 0.0022$) and diet ($P < 0.0001$) for BW at 42d in both trials. Both lines had a similar starting FSR (>40%d) and FBR (≈35%d) of mixed skeletal muscle protein for the start of the studies at d22. Line A had a higher FSR and FBR in the response to ME (17.01%d and 13.22%d) while Line B had a significantly higher FBR of 15.27%d and FSR of 18.31%d for skeletal muscle at 42d in the AA trial. FSR and FBR tended to increase as gdLys/Mcal increased in the diets, with the exception of the lowest ME level diet. Delta FBR revealed a significant line × diet interaction ($P = 0.0204$) in the AA trial. There was a significant 7.29%d FSR and 7.15%d FBR difference between the lowest and highest energy diets at 42d, ($P = 0.0002$, $P = 0.0005$) The AA trial suggests that FSR increased 6.73%d from the 80% AA dietn up to the 110% AA diet (20.44%d) at 35d. These trials suggest both strains synthesize and degrade more protein when fed increasing levels of amino acids per Mcal. Energy deficient diets (2800 kcal ME) can significantly limit muscular synthesis and degradation even if the gdigLys/Mcal is higher.

Key Words: protein turnover, broiler, infusion, synthesis, degradation

12 Ideal ratio of valine for Ross 308 male broiler chicks in growing period. A. H. Seyedi*[1] and E. Safiyary[2], *[1]Tabriz University, Tehran, Iran, [2]Uremia University, Tehran, Iran.*

A bioassay was conducted to determine the ideal ratio of digestible valine relative to lysine (dig Val/Lys) for male Ross 308 broiler chicks from 8 to 21 d of age. Valine-deficient corn-soybean meal basal diets (3030 MEn kcal/Kg) contained 20.8% crude protein and 0.70 ideal ratio dig Val/Lys for experimental period. To evaluate ideal ratio of dig Val/Lys of male broiler chicks based on growth performance, a total of 600 male broiler chicks were assigned to 6 treatments with 5 replicate using a completely randomized design. Dietary ideal ratio of dig Val/Lys was supplied in 6 treatments from 0.70 to 0.95 for the experimental period. Straight broken line Regression analysis indicated that ideal ratio of dig Val/Lys at 0.80 in diet maximized body weight gain; whereas 0.79 improved feed conversion ratio and 0.90 minimized the feed intake. Quadratic broken line Regression analysis showed that ideal ratio of dig Val/Lys at 0.86 of the diet maximized body weight gain, whereas 0.84 optimized feed conversion ratio. Feather free body weight, all analyzed body composition parameters and protein retention in body were significantly affected by the increasing ideal ratio of dig Val/Lys in basal diet ($P < 0.01$). Broiler chickens fed with 0.85 of ideal ratio of dig

Val/Lys had the maximum protein retention ($P < 0.01$). Results indicate that a ratio of dig Val/Lys of 0.86 should be adequate for improving Ross 308 broiler chick's performance.

Key Words: broiler, broken line, valine, weight gain

13 Effects of dietary energy and amino acid density during finisher and withdrawal phases on live performance and carcass characteristics of male and female Cobb MV × 700 broilers. C. Maynard*[1], R. Latham[2], R. Brister[2], C. Owens-Hanning[1], and S. Rochell[1], *[1]University of Arkansas, Fayetteville, AR, [2]Tyson Foods Inc., Springdale, AR.*

Two experiments (Exp) were conducted to investigate the interactive effects between dietary energy density and sex or amino acid (AA) density in Cobb MV × 700 broilers from 29 to 46 d post-hatch. In both Exp, broilers were reared to 28 d on common starter and grower diets before being provided experimental finisher and withdrawal diets. Low (LE), medium (ME), and high energy (HE) finisher diets (29 to 36 d) were formulated to contain 3,140, 3,175, and 3,210 kcal/kg AME_n, and LE, ME, and HE withdrawal diets (37 to 46 d) were formulated to contain 3,175, 3,210, and 3,245 kcal/kg AME_n, respectively. In Exp 1, 6 treatments included a factorial arrangement of dietary energy density (LE, ME, and HE) × sex. In Exp 2, 6 treatments included a factorial arrangement of dietary energy (LE, ME, and HE) × 2 levels of AA density. High (HAA) and medium AA (MAA) diets were formulated to contain digestible Lys concentrations of 1.00 and 1.07% in the finisher and 0.94 and 1.01% in the withdrawal phases, respectively. Ratios of other AA relative to Lys were held constant within each phase. Both Exp included 12 replicate pens of 12 birds. Body weight gain (BWG), feed intake (FI), and FCR were determined for each phase and cumulatively. At 47 d, 4 birds per pen were processed for evaluation of carcass characteristics and scored for woody breast (WB) and white striping in *Pectoralis major* fillets. All data were subjected to a 2-way ANOVA and differences were separated using Tukey's test. No effects ($P > 0.05$) of dietary energy or energy × sex interactions were observed on overall live performance or carcass characteristics in Exp 1. Males had higher ($P < 0.01$) BWG and FI and lower ($P < 0.01$) FCR than females throughout the trial. Male broilers had higher ($P < 0.01$) parts weights and incidence of severe WB than females. In Exp 2, no dietary energy × AA interactions were observed for any measurement, and dietary energy had no effect ($P > 0.05$) on overall FI, BWG, or FCR. From 29 to 46 d, birds fed the MAA diets had higher ($P < 0.01$) FI and BWG, but similar ($P > 0.05$) FCR, compared with birds fed the HAA diet. Increasing AA density from MAA to HAA increased ($P < 0.05$) tender yields but did not influence other parts weights or yields. Dietary energy levels influenced ($P < 0.05$) the incidence of slight WB but did not ($P > 0.05$) affect the incidence of normal or severe WB. Decreasing AA density from HAA to MAA decreased ($P < 0.05$) the incidence of normal WB without influencing ($P > 0.05$) slight or severe WB. In conclusion, Cobb MV × 700 broilers did not respond to the dietary energy levels assessed in the current Exp. Moreover, compensatory FI of broilers was more responsive to reduced AA density than to reduced dietary energy.

Key Words: amino acid, energy, broiler, performance, myopathies

14 Effects of all-vegetable and animal protein supplements for Hy-Line W-36 pullet development and layer performance. K. Foltz*[1], K. Karges[2], M. Hashim[2], and M. Persia[1], *[1]Virginia Tech, Blacksburg, VA, [2]H.J. Baker & Bro. LLC, Shelton, CT.*

The objective of the current study was to evaluate the use of vegetable and animal protein supplements (VeGain and Pro-Pak 60, H. J. Baker & Bro. LLC, Shelton, CT) in corn, soybean meal, and corn distillers dried grains with solubles (DDGS)-based pullet and layer rations. Three commercial-type diets were formulated based on specific ingredient analyses to be isocaloric and similar in digestible amino acids. Diets consisted of a corn/soybean meal-based diet (C/SBM) and C/SBM with 5% inclusion of Pro-Pak 60 (APS) or VeGain (VPS), while corn DDGS inclusion was held constant within diet phase. Dietary treatments were fed to 5 groups of 32 Hy-Line W-36 pullets from day-of-hatch through wk 4, 10 groups of 14 pullets from wk 4–8, 15 groups of 8 pullets from wk 8–16, and 16 groups of 6 hens for the remainder of the experiment. Feed refusal was measured each week and individual (wk 4–16) or group (wk 18–40) body weights were recorded every 4 weeks for performance and uniformity determinations. Over the layer phase (wk 18–40), egg production was recorded daily, while egg components and excreta moisture were determined every 4 weeks, and hen body composition was analyzed on wk 40. Pullet data was analyzed by one-way ANOVA using the GLM procedure and layer data by repeated measures ANOVA using the MIXED procedure in SAS 9.4 and LSMEANS were separated using Fisher's LSD and Tukey's HSD, respectively. Alpha level was set at 0.05 for all analyses. Average daily feed intake of pullets fed diets with APS was 1.1 g/day higher ($P < 0.01$) than those with VPS or C/SBM from wk 11–12 but were otherwise similar among treatments. Body weights were either similar or greatest ($P \leq 0.05$) for pullets fed diets with APS (wk 2, 4, and 12), though FCR was not different at any time point. Flock uniformity and body weight CV were improved (9.6% increase and 38% reduction, respectively) for pullets fed C/SBM or VPS in comparison to those fed APS at wk 16 ($P \leq 0.02$). During the layer phase, there were no significant treatment effects on egg production or performance ($P > 0.05$). Hens fed VPS or APS had 8.7 and 10% higher body fat mass than hens fed C/SBM on wk 40 ($P = 0.02$). Feeding diets with either APS or VPS also reduced excreta moisture by 2.5 and 2.4% compared with hens fed the C/SBM diet ($P = 0.01$). These data suggest that, within the current study, feeding diets with animal protein may increase BW, but may also reduce uniformity compared with pullets fed all-vegetable based diets. Additionally, diets containing animal or vegetable protein supplements reduced layer excreta moisture, while maintaining similar performance and egg quality characteristics.

Key Words: layer performance, vegetable protein, animal protein, excreta moisture, uniformity

15 Black soldier fly larvae meal and oil can replace soybean meal and oil without adversely affecting chick growth performance during the starter phase. B. Wall*[1], K. Livingston[1], E. Koutus[2], and C. C. Parsons[3], *[1]North Carolina State University, Raleigh, NC, [2]Enviroflight, Yellow Springs, OH, [3]University of Illinois, Urbana-Champaign, IL.*

Black soldier fly larvae (BSFL), *Hermetia illucens*, is a possible alternative protein (meal) and fat (oil) source, however the digestibility of these products needs to be elucidated. In experiment 1, we determined the true metabolizable energy (TMEn) of the whole BSFL and BSFL meal (BSFLm) using intact roosters. The roosters were fasted for 24 h and fed 30 g of BSFLm to 4 individual birds. Excreta samples were collected 48h later then freeze-dried, and TMEn determined. It was determined that the whole BSFL had TMEn of 4.545 ± 0.209 kcal/g DM, while BSFLm had a lower TMEn of 3.334 ± 0.037 kcal/g DM ($P < 0.001$). In experiment 2, titrated levels of BSFLm (0, 5, 10, and 15%) were included broiler diets at the expense of SBM. In experiment 3, titrated levels of BSFL oil (BSFLo; 0, 0.5, 1.5, 3%) were added at the

expense of soybean oil. The TMEn was based on previously published values of 8.500 kcal/g. In experiments 2 and 3, chicks were reared in Petersime cages with 7 chicks per cage and 5 replicates per treatment. Chick weight, and feed intake were measured weekly and feed conversion ratios (FCR) were calculated. Data were analyzed as a one-way ANOVA using Sigmaplot ®. No differences were observed in BWG, feed intake, FCR, or flock uniformity regardless of the inclusion level of BSFLm (exp 2), or BSFLo (exp 3) ($P > 0.05$). In conclusion, BSFLm was determined to have a TMEn of 3.334 kcal/g which was able to replace SBM up to 15% without any deleterious effects. Moreover, BSFLo can replace soybean oil up to 3% without any adverse effects on chick performance during the starter phase.

Key Words: black soldier fly larvae, broiler, digestibility, starter

16 Effects of feed form and amino acid densities on productive and processing performance of broilers. A. Rubio*, A. Lopez, J. Hess, W. Berry, W. Dozier, III, and W. Pacheco, *Auburn University, Auburn, AL.*

Previous research has reported improved growth performance in birds fed 4.4 mm pellets compared with mash diets. Currently, the majority of broiler producers are feeding crumbles during the starter phase and pellets in subsequent periods. However, a processing alternative could be feeding 3.3 mm micro pellets during the whole production period. Amino acid (AA) density also has an impact in growth responses and meat accretion of broilers. However, research evaluating the interactive effects between feed form and AA density throughout the production period of broilers is sparse. The objective of this study was to evaluate the effects of feed form and dietary AA densities on growth and processing performance of broilers. One thousand 1-d old male Ross × Ross 708 broiler chicks were randomly distributed among 6 treatments with 7 replicates pens per treatment, which constituted a 3 × 2 factorial arrangement of 3 feed forms (mash, 4.4 mm pellets, and 3.3 mm micro pellets) and 2 AA densities (88% and 96% of Aviagen recommendations) provided from 1 to 42 d of age. Diets were offered as crumbles from 1 to 14 d on birds fed 4.4 mm pellets. Feed consumption and BW were determined at 14, 28 and 42 d of age and adjusted feed conversion (FCR) was calculated by using the weight of the mortality. At 43 d, 10 birds/pen were processed for the determination of carcass characteristics. At 44 d, chilled carcasses were deboned to determine total breast meat yield (pectoralis major and minor muscles). Data were statistically evaluated as a 3 × 2 (feed form × dietary AA) factorial arrangement in a randomized block design. Interactions were not ($P \geq 0.05$) apparent for the variables tested in this study. Birds fed 3.3 mm micro pellets and 4.4 mm pellet had higher BW, FI, carcass weight, and breast meat weight and better feed conversion (FCR) than birds fed mash diets at 42 d of age ($P \leq 0.05$). In addition, birds fed diets with 96% of AA recommendations had higher BW, carcass yield, carcass weight, and breast meat weight and better FCR than birds fed diets with 88% of AA recommendations ($P \leq 0.05$). These data indicated that broilers can be fed 3.3 mm micro pellets and 4.4 mm pellets and 96% AA density during the starter period and/or during the whole production period of broilers to improve growth performance and meat accretion at 42 d of age.

Key Words: amino acid, carcass, crumble, micro pellet, pellet

17 Feed intake, egg production, eggshell quality, and apparent retention of nutrients in Lohmann Select Leghorn and Shavers Heritage hens fed high fiber diets. A. W.-Van Humbeck* and E. Kiarie, *University of Guelph, Guelph, ON, Canada.*

Micronutrients, such as Ca, have several factors impacting their ability to travel from feed to eggshell, bone or excreta. Among others, factors influencing nutrient utilization include feed composition and genetics. By adding ground (GOH) or unground (UGOH) oat hulls in diets of 57-wk old Lohmann Select Leghorn-Lite (LSL) and 44-wk old Shaver Heritage White Leghorn (SHWL) hens, we investigated the impact of diet structure and processing on feed intake, egg production, eggshell quality and apparent retention (AR) of nutrients. Diets fed were either commercial layer mash, or a mixture of commercial layer mash with GOH or UGOH at a ratio of 80:20 wt/wt. TiO_2 was used as an indigestible marker. A total of 288 birds (144/breed) were placed within breed in cages (6 hens/cage), fed a control diet for 1-wk to collect egg production data as basis for diet allocation, and subsequently allocated diets to give 8 replicates. Diets were fed for 28 d, during which hen day egg production (HDEP) was recorded daily and feed intake bi-weekly. Measures of egg quality (egg weight, EW, egg shell thickness, EST and breaking strength, EBS) were performed on all eggs laid on d 26–28. Excreta samples were collected on d 25–28 and pooled by cage for analyses. Data was analyzed using JMP 13.1 in a 2-way ANOVA with fixed effects of diet, breed and their 2-way interaction. There was an interaction ($P < 0.01$) between diet and breed on HDEP in weeks one and 2 such that the feeding of oat hulls reduced HDEP in LSL but not in SHWL. There was no ($P > 0.05$) diet and breed interaction on HDEP in wk 3 and 4, main effects were such that hens fed UGOT showed ($P < 0.01$) lower HDEP in wk 3 compared with hens fed other diets, however, there was no ($P > 0.05$) diet effect on HDEP in wk 4. Egg weight and FCR (egg mass/feed intake) were higher ($P < 0.01$) for LSL compared with SHWL. There were no effects ($P > 0.05$) on feed and energy intake. An interaction between diet and breed ($P < 0.01$) was seen on EST and EBS, EST was lower in LSL birds fed GOT and in SHWL birds fed UGOT relative to respective control birds. Generally, LSL had higher ($P < 0.01$) EST and EBS than SHWL birds. Birds fed oat hulls had lower ($P < 0.01$) AME relative to the control however, birds fed UGOH had higher AME (2,962 vs. 2,758 kcal/kg DM, $P < 0.01$) than birds fed GOT. In summary, better performance of LSL indicates genetic improvement over time. By wk 4 there was no diet effect on HDEP due to oat hulls, however poor eggshell and nutrient utilization was evident. Feeding UGOT improved eggshell and AME relative to GOT perhaps related to positive effects on gut physiology in modern birds.

Key Words: layer, fiber, processing, production, eggshell quality

18 Effects of feeding a mixture of flaxseed and pulses (linPRO) with or without multi-carbohydrases supplement on egg yolk fatty acids composition and liver weight in broiler breeders. A. Thanabalan*[1], J. Moats[2], and E. Kiarie[1], [1]*University of Guelph, Guelph, ON, Canada,* [2]*O&T Farms Ltd., Saskatoon, SK, Canada.*

Flaxseed products have been proposed as a potential strategy to enrich broiler breeder (BB) eggs with omega-3 fatty acids to benefit the progeny. However, there is limited information on yolk fatty acid (FA) enrichment in feed-restricted BB. Moreover, maintaining liver health is critical in BB feeding management and it is imperative to investigate whether flaxseed products impact liver physiology. The objective of this study was to determine egg yolk FA composition and liver weight in BB fed a dry-extruded flaxseed product (linPRO, a mixture of full-fat flaxseed and ground pulses (1:1 wt/wt) with or without a multi-carbohydrase (MC) supplement. The MC supplied 1,200 U of xylanase, 600 U of β-glucanase, 2800 U of cellulase, 400 U of mannanase, and 2,500 U of amylase per kg of feed. A basal corn-soy based diet was formulated to meet the breeder specifications. Test diets were made by mixing the

basal diet with linPRO in a 82:18 wt/wt ratio. Twenty-six week old Cobb 500 BB were allocated to 30 identical cages (2 hens/cage) and given a one week adaptation period to the cages. The 3 diets were randomly assigned to 10 replicate cages based on BW after adaptation. Birds were fed based on breeder curve for 30 d. Eggs were collected from d 28–30 consecutively, and yolks were separated, weighed, pooled by cage, and frozen until analysis. All birds were killed on d 30, and livers removed, weighed, and freeze-dried. Frozen yolks were lyophilized to determine dry yolk weight. Dried yolk samples were hydrolyzed with methanol and FA concentrations analyzed using gas chromatography. Dried liver and yolk weights as well as total yolk FA were not influenced by treatments ($P > 0.05$). Birds fed the control diet had lower concentration of α-linolenic acid (C18:3n-3) than birds fed linPRO with or without enzymes ($P < 0.0001$). Specifically, the concentration of C18:3n-3 was 7.5, 36.8 and 37.3 mg/g dried yolk in the control linPRO without and linPRO with MC, respectively. Furthermore, birds fed control diet displayed lower concentrations of docosahexaenoic acid (C22:6n-3) than birds fed linPRO with or without MC ($P < 0.001$). The concentrations of C22:6n-3 were 4.1, 9.1 and 8.5 mg/g of dried yolk in control, linPRO without and with MC, respectively, indicating higher conversion of C18:3n-3 to C22:6n-3 in birds fed linPRO. Finally, the omega 6:3 ratio in the control yolks was higher than birds fed linPRO with or without MC ($P < 0.001$). The study indicated that feed-restricted BB enriched egg yolks with omega-3 FA from flaxseed products without adverse effects on the liver. The inclusion of a MC did not affect the deposition of fatty acids within linPRO treatments.

Key Words: linPRO, broiler breeder, omega-3, liver weights, enrichment

Student Competition: Metabolism and Nutrition, Vitamins and Minerals I

19 Evaluation of increasing levels of copper hydroxychloride in corn/soy diets on male broiler performance through 49 days. K. Smith*[1], A. Jasek[1], T. Parr[2], and J. Lee[1], *[1]Texas A&M University, Jarrell, TX, [2]Micronutrients USA LLC, Indianapolis, IN.*

The objective of the current study was to evaluate the influence of increasing levels of copper hydroxychloride (IntelliBond CII) in a corn/soy diet on broiler growth performance. Experimental treatments were manufactured from 1 basal diet formulated to replicate a typical industry broiler ration utilizing a mineral premix without copper. The control was the basal diet with the addition of 5 ppm of copper sulfate to meet the nutritional requirement of broiler chickens while the other 3 dietary treatments consisted of the copper-free basal with the addition of copper hydroxychloride at 100, 200 and 300 ppm. Each treatment included 10 replicate pens with 35 Ross 708 males placed per pen. The dietary program consisted of 4 phases including the starter through d 14, grower through d 28, finisher through d 42 and withdrawal through d 49. The experimental design was conducted as a one-way ANOVA. Additionally, linear and quadratic regression was used to evaluate the impact of increasing levels of copper hydroxychloride on broiler performance. Data were collected as feed consumption (FC) and BW gain (BWG) to coincide with phase changes at d 14, 28, 42 and 49. Throughout the experiment, copper hydroxychloride inclusion at 200 and 300 ppm improved ($P < 0.05$) FC compared with the control. The increases observed in FC were responsible for increases ($P < 0.05$) in BW and BWG compared with the control diet. Body weight gain was elevated as with increasing levels of copper in each dietary phase ($P < 0.05$). Feed conversion ratio (FCR) was also improved ($P < 0.05$) in broilers fed 200 and 300 ppm copper hydroxychloride as compared with the control. However, further improvements ($P < 0.05$) were observed with the inclusion of copper hydroxychloride at 300 ppm compared with 200 ppm in BW at d 42 and 49, cumulative FCR from d 0–49, and FC from d 28–42. Linear regression and quadratic regression analysis indicated significant increases in BW and reduction in cumulative FCR with increasing Cu. In conclusion, these data support the idea that utilizing copper hydroxychloride in broiler diets can lead to performance improvements in FC, FCR, BW and BWG and improvements continue to be observed through an inclusion rate of 300 ppm.

Key Words: copper, hydroxychloride, broiler, performance

20 Growth performance and carcass characteristics of broilers fed diets varying in amino acid densities and supplemental copper concentrations from 1 to 33 days of age. S. Philpot*[2], K. Perryman[1], K. Macklin[2], and W. Dozier, III[2], *[1]Micronutrients, Indianapolis, IN, [2]Auburn University, Auburn, AL.*

Preliminary research indicates that feeding concentrations of Cu above nutritional requirements may enhance amino acid (AA) digestibility, which could support growth performance in broilers fed low AA diets. An experiment was conducted to determine the interactive effects of dietary AA density and supplemental Cu concentrations on growth performance and carcass characteristics of Ross × Ross 708 broilers. Male chicks were randomly distributed to 64 floor pens (25 birds per pen; 0.09 m^2/bird). A 2 × 3 factorial arrangement of 2 dietary AA densities and 3 Cu programs with positive (PC) and negative (NC) controls was employed, with 8 replicate pens per treatment. Moderate (Mod) and Low AA diets contained 95 and 88% of the recommended density in the 2014 Ross 708 Broiler Nutrition Specifications, respectively. Supplemental Cu was provided in 3 programs in the form of tribasic copper chloride (Micronutrients USA, LLC), where the Half program received 135 ppm of supplemental Cu in all phases, the Full program contained 270 ppm in the starter and grower phases and 135 ppm in the finisher phase, and the Step-Down program contained 270 ppm in the starter phase and 135 ppm in the grower and finisher phases. Positive control and NC birds were fed Mod AA diets with approximately 13 ppm Cu. In addition, PC broilers were unvaccinated and received diets containing diclazuril, whereas all other birds received Coccivac B52 at hatch. Broilers were fed a 3-phase feeding program with corn, soybean meal, and DDGS as the primary ingredients. Body weight gain (BWG), feed intake (FI), feed conversion (FCR), and mortality were determined at 12, 25, and 32 d of age. At 33 d, 10 birds per pen were processed and deboned to determine weights and yields of parts. Data were analyzed for main and interactive effects and with preplanned orthogonal contrasts. No interactive effects were observed in cumulative performance ($P > 0.05$). Broilers fed Mod AA diets had higher BWG and lower FCR at 32 d than birds fed Low AA diets ($P < 0.001$). Birds fed Mod AA, Full Cu diets had lower cumulative FI and FCR (1.42 vs. 1.44) ($P \leq 0.04$) than those fed Mod AA, Half Cu diets. Broilers fed Mod AA diets containing the Full and Step-Down Cu programs also had lower cumulative FI and FCR (1.42 and 1.43 vs. 1.45) than NC fed birds ($P \leq 0.025$). There were no cumulative differences in BWG based on Cu program ($P > 0.05$). Total breast meat weight and yield was higher for birds fed Mod AA diets than those fed Low AA diets ($P < 0.05$) but was not affected by Cu programs ($P > 0.05$). These results indicate that feeding high concentrations of supplemental Cu to broilers may improve growth performance but does not necessarily compensate for decreased AA density.

Key Words: amino acid, antibiotic free, broiler, copper, vaccine

21 Relative bioavailability of humate-manganese complex for broilers fed a corn-soybean meal diet. S. Bai*[1], X. Ding[1], K. Zhang[1], J. Wang[2], Q. Zeng[1], and H. Peng[1], *[1]Animal Nutrition Institute, Sichuan Agricultural University, Chengdu, Sichuan, China, [2]Sichuan Agricultural University, Chengdu, China.*

The experiment was conducted to investigate the relative bioavailability of humatic- manganese (Mn) to Mn sulfate for broilers fed a conventional corn-soybean meal diet. A total of 560 1-d-old Arbor Acres commercial male chicks were fed the Mn-unsupplemented basal diet or the basal diet supplemented with 60, 120, or 180 mg Mn/kg from each Mn source with 8 replicates each treatment (10 birds per replicate cage). At 14 d of age, the plasma, liver, heart, and tibia were collected from one bird each replicate cage. The Mn concentration in different tissue, and the activity and mRNA abundance of heart manganese superoxide dismutase (MnSOD) were determined. The Mn concentration in the plasma, tibia, and heart, and the activity and mRNA abundance of heart MnSOD increased ($P < 0.05$) linearly with dietary Mn concentration increasing. Based on slope ratios from multiple linear regression of Mn concentrations in the plasma and heart, and heart MnSOD mRNA abundance on added Mn level, there was a trend of low bioavailability ($P < 0.10$) in humatic-Mn complex as compared with Mn sulfate (80.8% vs 100%) for broilers from d 1 to 14.

Key Words: manganese, broiler

22 Effects of pre-lay dietary calcium and strain on age, body-weight, femur weight, ash retention and calcium intake at first egg in pullets. T. Khanal*, T. Widowski, G. Y. Bédécarrats, and E. Kiarie, *University of Guelph, Guelph, ON, Canada.*

Advances in genetic selection of laying hens have reduced the age, feed intake and body weight (BW) at sexual maturity necessitating further research in optimal Ca nutrition during this period. We investigated the effects of pre-lay diet Ca level and strain on age, BW, femur weight (FW), apparent ash retention (AR) and Ca intake at first egg in pullets. Thirty Lohmann Brown (LB) and 30 Lohmann LSL-Lite (LSL) pullets (14 wk of age, woa) reared under same management regimen (housing and diet) were procured from the University of Guelph Arkell station flock. Six pullets per strain were randomly selected and sacrificed for baseline femur samples. The remaining 48 pullets were housed individually in cages (65 cm × 30 cm × 45cm) and fed 1% Ca developer diet for 2 wks (14–15 woa) of adaptation period. At the start of 16 woa, pullets were weighed and allocated within strains (LB vs. LSL) to pre-lay diets (2.5 vs. 4.0% Ca) effectively creating a 2 × 2 factorial design (n = 12). The birds were offered pre-lay diets for 2 wk (16 −17 woa) and switched to layer diet (4% Ca) at 18 woa. The diets contained TiO_2 as indigestible marker to determine AR. Photoperiod was set at 10 h (4–6 lx) from 14 to17 woa and then increased 1 h per week until 14 h at 20 woa with an intensity of 10–15 lx. The age, BW and feed intake were recorded, excreta samples were taken, and 4 hens per treatment were sacrificed for femur samples at first egg. The interaction and main effects of factors on response variables were identified by using 2-way ANOVA. The total feed and Ca intakes from pre-lay to first egg were calculated. Pre-lay dietary Ca did not interact ($P > 0.05$) with strain on any response criteria. Pre-lay dietary Ca had no ($P > 0.05$) effect on age, BW, FW, AR, or total feed intake at first egg. However, Ca intake was higher (~20.81%, $P < 0.05$) for hens fed the pre-lay diet with 4% Ca. Lohmann Brown pullets laid their first-egg earlier (116.8 vs. 126.1 d, $P < 0.01$) than LSL-Lite pullets. The LB pullets had higher BW both at 14 woa (1,491 vs. 1,078 g, ~38.3%, $P < 0.001$) and at first egg (1,791 vs. 1,485 g, ~20.6%, $P < 0.001$) than LSL. In contrast, relative FW for LB and LSL was similar at first egg (5.14 vs. 5.07 mg/g BW, $P = 0.521$). The LSL pullets ate 40.6% more feed (1,519 vs. 1,080 g, $P = 0.01$) and 48.4% more Ca (61.5 vs. 41.4 g, $P = 0.01$) than LB at the time of first egg. Also, LSL retained 51.4% more ash (79.6 vs. 52.7 g) than LB at first egg. In conclusion, Pre-lay dietary Ca had no influence on FW, BW, AR and age at first egg. However, LSL pullets laid first egg when relative FW was similar to that of LB pullets.

Key Words: calcium, bone, femur, strain, hen

23 Effect of 1-α-hydroxycholecalciferol on grower diets with different levels of calcium on live performance and meat quality of Ross 708 broilers. V. San Martin*[1], E. Oviedo-Rondón[1], H. Cordova-Noboa[1], J. Cifuentes[1], C. Florez[1], F. Tovar[1], and J. D. Fernandez[2], *[1]North Carolina State University, Raleigh, NC, [2]Premex Inc., Medellin, Antioquia, Colombia.*

Active vitamin D_3 is an important nutrient involved in overall physiology, especially for Ca absorption and metabolism. Previous studies indicated that $1\alpha(OH)D_3$, a metabolite of Vit D_3, ameliorated the negative effects of low dietary Ca on FCR during the first 14 d. However, at higher (1.40%) Ca levels the retention of minerals, like Ca and P, tends to reduce and performance is affected. One experiment was conducted to evaluate the effects of $1\alpha(OH)D_3$ supplementation during the grower phase (16–34 d) in diets containing variable levels of Ca on broiler live performance, flock uniformity and meat quality parameters. A total of 1,152 male Ross-708 one-day-old chicks were placed in 48 pens with 24 chicks each. One starter diet containing $1\alpha(OH)D_3$ was fed for all chickens from 1 to 16 d. At 17 d of age, 8 treatments generated from a factorial arrangement of 4 levels of dietary Ca (0.54; 0.76; 0.98 and 1.20%), and the supplementation or not of $1\alpha(OH)D_3$ (0 *vs*. 5µg/kg feed) were offered. Grower dietary treatments were obtained from a basal diet and Ca content was adjusted by including limestone, dicalcium phosphate and sand depending on the treatment. All diets contained phytase providing 0.12% of available P and 0.12% of Ca. BW and feed intake were obtained, and BW gain and FCR were calculated at 16 and 34 d of age. Six broilers per pen were selected and processed. Carcass and cut-up yields were obtained and whole *Pectoralis major* muscle was used to determine meat quality parameters. Data were analyzed in a randomized complete block design with dietary Ca level and $1\alpha(OH)D_3$ supplementation as main factors and pen lines were considered as block random effect. Two-way ANOVA, mean separation by Tukey's and *t*-test, and regression analyses were conducted. At 34 d no interaction or main effects ($P > 0.05$) were detected on BW, BW gain or flock uniformity. For FCR, the regression analysis indicated a quadratic effect due to Ca levels ($P < 0.05$), in which the best FCR was estimated at 0.91% Ca. In processing results, quadratic effects ($P < 0.001$) of Ca levels on hot and cold carcass yield were detected. Regression analysis for both variables indicated higher carcass yields at 0.90% Ca level. On breast meat quality parameters, effects of Ca were observed ($P < 0.05$) in cook loss and color lightness value. No effect ($P > 0.05$) was observed in woody breast or white stripping scores; however, spaghetti muscle increased ($P < 0.05$) with 0.54% Ca level. In conclusion, no consistent effects of $1\alpha(OH)D_3$ inclusion were observed during the grower phase, when starter diets contained $1\alpha(OH)D_3$. It was confirmed that Ca levels during the grower phase play an important role on live performance, carcass yield, and meat quality parameters.

Key Words: 1-alpha-vitamin D3, calcium, performance, carcass traits, meat quality

24 Effects of the in ovo injection of vitamin D_3 and 25-hydroxyvitamin D_3 on meat yield and woody breast incidence in broilers fed commercial or calcium and phosphorus-restricted diets. S. Fatemi*[1], K. E. C. Elliott[1], W. Zhai[1], A. Bello[2], B. Turner[3], and E. D. Peebles[1], *[1]Mississippi State University, Starkville, MS, [2]University of Alberta, Edmonton, AB, Canada, [3]DSM Nutritional Products, Parsippany, NJ.*

Broiler performance and meat yield has been improved by the use of supplemental dietary vitamin D_3 (D_3) and its metabolite, 25-hydroxyvitamin D_3 ($25OHD_3$). However, effects of the in ovo injection of D_3 and $25OHD_3$ on meat yield and quality are not well understood. Therefore, the objectives of this study were to determine effects of the in ovo administration of D_3 and $25OHD_3$ on broiler performance, carcass characteristics, and incidence of woody breast myopathy (WBM). Live embryonated Ross 708 broiler hatching eggs were randomly assigned to one of the following 4 in ovo injection treatments at 18 d of incubation (doi): 1) diluent (control); diluent containing either 2) 2.4 µg D_3; 3) 2.4 µg $25OHD_3$; or 4) 2.4 µg D_3 + 2.4 µg $25OHD_3$. A 50 µL solution volume was injected to each egg using an Inovoject multi-egg injector. At hatch, 18 male chicks were randomly placed in each of 48 floor pens. Beginning on d of hatch, in each of 6 replicate pens belonging to each in ovo injection treatment group, birds were fed either a commercial diet or a diet restricted in calcium and phosphorus (ReCaP) content by 20% for the starter, grower and finisher dietary phases. Average daily BW gain (ADG) and feed intake (ADFI) as well as feed conversion ratio (FCR) were determined weekly from 0 to 45 d of age (doa). Pectoralis major and minor muscle (PEC), bursa, liver, spleen, duodenum, jejunum, and

ileum weights were measured at 1, 7, 14, and 40 doa. At 41 and 46 doa, birds were also processed for determination of WBM, carcass weight, and the absolute and relative (% of carcass weight) weights of carcass parts. Main and interaction effects of injection and dietary treatments were analyzed using 2-way ANOVA, with significance set at $P \leq 0.05$. Compared with birds fed the commercial diet, birds fed ReCaP diets had a significantly lower ADG ($P = 0.001$), ADFI ($P = 0.001$), and FCR ($P = 0.001$) from 7 to 45 doa, and a lower PEC ($P = 0.001$) from 14 to 46 doa. The WBM ($P = 0.001$) was also lower in ReCaP-fed birds compared with the commercial-fed birds. Although, treatment did not affect organ weight, at 14 and 40 doa, birds that received in ovo treatment 3 displayed a higher PEC yield ($P = 0.003$) when compared with birds that received in ovo treatments 1 or 4. The lower WBM score in the ReCaP-fed birds may be associated with lower breast weight, and an increase in PEC yield in birds belonging to in ovo treatment 3 may be due to an improvement in broiler immunity or small intestine morphology which may result in increased nutrient absorption. However, effects of the in ovo injection of various vitamin D_3 sources on small intestine morphology and the immune status of broiler chickens deserve future investigation.

Key Words: in ovo administration, 25-hydroxyvitamin D_3, calcium and phosphorus-restricted diet, broiler performance, woody breast

25 Histopathological comparison between three different methods for euthanasia of broiler chickens. A. T. Silva*, J. Wammes, A. Sanches, B. Belote, I. Soares, B. Lunardeli, J. Duque, and E. Santin, *Federal University of Paraná, Curitiba, Brazil.*

Few data are available regarding methods of euthanasia of chickens for experimental analysis. The euthanasia should be humane, therefore quick, effective and trustable for the bird to lose its conscience and die quickly, with the least pain and suffering possible. The aim of this study was to evaluate the histological alterations caused by 3 different methods of euthanasia. An amount of 24 21 d-old Cobb 500 chickens were divided into 3 different groups (G), being G1 – euthanized with cervical dislocation; G2 – with intravenous xylazine 20 mg/kg and ketamine 20 mg/kg followed by administration of lidocaine 2 mL of 2% intrathecal; G3 – also with intravenous xylazine 20 mg/kg and ketamine 20 mg/kg followed by administration of 2 mL of KCl 19.1%. After confirmed death by the absence of heart beating, samples of brain, heart, ileum, liver and kidney were gathered, fixed with neutral buffered formalin 10% and stained with hematoxylin-eosin for histopathological analysis regarding acute lesions linked to hypoxia and anoxia. The lesions were graded from zero to 3 according with the severity, being the score 3 the most severe and wide parameters. The mean value of the analysis for each animal was submitted to Shapiro-Wilk normality test. Rates were compared using one-way ANOVA followed by Tukey test ($P < 0.05$). In the liver, it was observed higher score of tumefaction on G2 (2.0) compared with other groups (1.1 for both G1 and G3). There was no significant difference for other parameters evaluated (congestion and necrosis). Regarding kidney and heart, the parameters evaluated were congestion, hemorrhage, necrosis and tumefaction. It was observed that G1 showed the highest and G2 the lowest value for congestion on kidney (1.1 for G1 and 0.6 for G2) and heart (1.4 for G1 and 0.6 for G2) compared with G3 that was intermediary (0.8 in kidney and 1.1 in heart). Regarding hemorrhage, G1 also showed the highest score in the heart (1.5) while G3 showed the lowest (1.0) compared with G2 that was intermediary (1.2). For the other parameters in kidney and heart and all parameters observed in intestine and brain, there were no significant differences. It was possible to conclude that the protocol using cervical dislocation caused the greatest congestion on kidney and heart, hemorrhagic on heart, but the lowest tumefaction on the liver compared with pharmacological methods. Although, for choosing the euthanasia method, the professional should evaluate according to the focus of the research, to pick the best method with lower alterations in the targeted organs.

Key Words: poultry, cervical dislocation, lidocaine, xylazine, ketamine

26 Assessment of aversion and time to insensibility to CO_2 gas exposure in laying hens. R. Bandara*[1], S. Torrey[1], R. Parsons[2], T. Widowski[1], and S. Millman[2], *[1]University of Guelph, Guelph, ON, Canada, [2]Iowa State University, Ames, IA.*

Carbon dioxide (CO_2) is often used for gas stunning/killing of poultry. For the method to be humane, it is essential to identify CO_2 concentrations which are not aversive to the species and cause rapid insensibility. The objective of this study was to examine the aversiveness of CO_2 to laying hens using approach-avoidance and conditioned place avoidance paradigms. A preference test was designed using 2 identical chambers separated by a sliding door and a curtain. The control chamber (CC) was maintained at ambient air conditions (1% CO_2) and treatment chamber (TC) at predetermined CO_2 concentrations. Twelve hens (1.20 ± 0.10 kg; 24 wks old) were individually trained for 5 consecutive days to enter the TC by pushing through a curtain to access rewards (feed and enrichment) with ambient air in both chambers. After entering TC, hens could move freely between chambers for 5 min, after which they were returned to the home pen. During the testing phase, the same procedures were used with the TC maintained at one of 4 CO_2 levels: 25%, 35%, 50%, or 70%. Tests were concluded after 5 min, or if loss of posture (LOP) occurred hens were immediately removed from the chamber for recovery. Hens were assigned to all CO_2 treatments (in random, then systematic, order), with each round of testing consisting of one gas days (G), preceded by one baseline day (B, 1% CO_2) and followed by one wash-out day (W, 1% CO_2). Behavior was collected using live observations, and analyzed using Fisher's exact test. Proportion of hens that entered TC on G days differ by treatment ($P = 0.007$, 25%CO_2 = 8/8; 35% CO_2 = 8/9; 50% CO_2 = 6/11; 70% CO_2 = 2/7). Pairwise comparisons showed that the proportion of hens that entered TC on G was different for 25% vs 50% ($P = 0.04$), 25% vs 70% ($P = 0.006$), and 35% vs 70% ($P = 0.034$). All the hens that entered TC on G showed LOP (mean latency: 15 ± 3 s). Conditioned place aversion was observed, since some hens that entered TC on G did not enter on W (25% CO_2 = 3/8; 35% CO_2 = 3/8; 50% CO_2 = 3/5; 70%CO_2 = 1/2). Some hens continued to avoid TC in subsequent rounds of testing. In conclusion, none of the laying hens tested actively avoid 25% CO_2, which resulted in LOP within less than 20 s. Individual differences in sensitivity appear to exist; some hens displayed conditioned place avoidance following LOP at any CO_2 concentration.

Key Words: aversiveness, laying hen, insensibility, CO_2, preference test

27 Perching behaviors of broilers affected by different perch designs. V. Nacchia* and H. Li, *University of Delaware, Wilmington, DE.*

In commercial houses broilers have limited active behaviors, which could cause leg problems. Some research suggests that adding perches can improve leg strength and mobility of broilers. However, the use of perches by broilers has been low and might be due to perch designs. We hypothesized that improving perch design could increase the use of the perches by broilers. Therefore, this study was conducted to investigate the effects of shape, height, and combinations of perches on perching behaviors. The objective of this study was to determine the perch design criteria based on the preferences of broilers. The study consists of 3 experiments. The first experiment was to compare the perches with 2 shapes (round vs. square) and 2 heights (4 in. and 6 in.) with a 2 × 2 experimental design. Four identical pens (2.25 ft × 5 ft) with 4 different perches were used and 4 birds were raised over a 6-wk. grow out period in each pen. Perching activities were monitored and recorded by load sensors and a computer program. Perching durations and step-on events were analyzed with a generalized linear model in JMP 12.0. In the second experiment, a preference test was performed with 6 birds in 2 choice pens. One pen was equipped with the most preferred perch from the first experiment and the other was without perch. Perching, locomotion, and feeding activities of broilers were monitored by load sensors and video cameras and recorded by computer programs. The preference of birds for perch was compared by a pairwise *t*-test. Feed consumptions in each pen were measured and correlated to the bird preference for perch. In the third experiment, 3 perch designs were further tested under field condition to verify the findings from the laboratory experiments.

Broilers performed more perching activities on a square shape ($P = 0.02$) and low profile perches ($P < 0.01$) than round shape and high profile perches. Perch height should be kept below 6 in. The results showed the reference of broilers for perch enrichment ($P = 0.003$). Broiler preferred simple perch design to the designs with high complexity.

Key Words: animal welfare, perch, broiler, choice, behavior

28 Effect of heat stress on serum biochemical responses and small intestinal integrity in ducks. X. Bu*, *Feed Science Institute, Hangzhou, Zhejiang, China.*

The present study was conducted to evaluate the effect of heat stress on stress related serum biochemical parameters and small intestinal integrity in ducks. A total of 120 Shaoxing ducks (60-d-old) with similar performance were kept in a temperature-controlled room with flowing air, sufficient water and ad libitum feeding during the experimental period. The ducks were allocated into 2 groups randomly with 4 replicates of 15 ducks. After 20 d of pretreatment period with room temperature of 25 ± 1°C before the hyperthermia challenge, the room temperature of heat stress group was increased at 10°C/h from 25 ± 1°C to 41 ± 1°C for 6 h (0900–1500 h) and 25 ± 1°C for the rest time, the control group were maintained at 25 ± 1°C throughout the treatment period (15 d) with sampling from each group on Days 1, 3, 5, 7, 10 and 15 respectively. Results were analyzed by one-way ANOVA and Student's *t*-test. Results showed that activities of serum aspartate aminotransferase (AST), creatine kinase (CK) and content of serum cortisol (COR) in heat stress group were 2.06-fold ($P < 0.01$), 1.66-fold ($P < 0.05$) and 16.62% ($P < 0.01$) higher than those of the control group on D10 respectively. Among the heat stress group, the activity of AST on D10 was higher ($P < 0.05$) than those on D3, D5, D7 and D15, meanwhile, the activity of CK and content of COR on D10 was the highest. The heat stress group had lower villus height as well as villus height: crypt depth ratio of duodenum on D7 ($P < 0.01$) compared with those of the control group respectively. Among the heat stress group, both villus height and villus height: crypt depth ratio on D7 were the lowest. Therefore, the current study indicated that the negative effects on serum biochemical homeostasis reached the highest on D10 when exposed to heat stress, while on small intestinal integrity, the negative effects reached the highest on D7.

Key Words: heat stress, duck, serum, biochemical parameters, small intestinal integrity

29 Pea starch increases night feeding behavior in laying hens. E. Herwig*, H. Classen, and K. Schwean-Lardner, *University of Saskatchewan, Saskatoon, SK, Canada.*

Replacement of wheat starch (WS) with graded levels of more slowly digested pea starch (PS) in laying hen diets increases feed intake and serum glucagon-like peptide-1 (GLP-1) while affecting serum peptide YY (PYY) in a quadratic fashion with a maximum at 50% PS. These 2 hormones are believed to be responsible for ileal brake activation, ultimately increasing satiety. It was hypothesized that activation of the ileal brake by the presence of starch in the distal small intestine of laying hens increases satiety by increasing GLP-1 and PYY and thereby alters feeding behavior. Two semi-purified starch sources with differing in vitro digestibility (WS, more rapidly digested and PS, more slowly digested) were used in 4 diets with identical starch content, but differing in the WS/PS ratio: 100/0, 80/20, 60/40, and 0/100. Diets were fed to 192 Lohmann LSL-lite laying hens from 26 to 46 weeks of age, housed in 16 experimental units. Each unit consisted of 2 conventional cages housing 6 hens each. Feed and water were offered at libitum. Two focal birds per experimental unit were individually marked and video recorded for 24h at 46 weeks of age. The initiation and end of every feeding bout were recorded and used to calculate: number of bouts (N), bout length (BL), time between bouts (TBB), and total time feeding (TT) during 24h, the photo- and scoto-periods, as well as the time from the start of the scoto-period to the first night feeding bout (T1N). A feeding bout ended when no more feeding occurred within 10s. Data were analyzed as a one-way ANOVA with SAS 9.4 GLIMMIX procedure, and Tukey's test was applied for mean separation when ANOVA indicated significant differences among treatments. Significance was considered when $P \leq 0.05$ and values are presented in order of increasing PS inclusion level. Superscripts indicate mean separation. Diet did not affect 24h or daytime feeding behavior; average values were n = 171, BL = 47s, TBB = 484s, and TT = 8545s for 24h and n = 170, BL = 47s, TBB = 275s, and TT = 8338s for the photoperiod. During the scoto-period, PS level affected both N (2.4b, 3.3ab, 3.8ab, 9.4a) and T1N (27398sa, 25586sb, 22059sc, 14609sd). Diet did not affect night TBB (average 2353s) or TT (average 208s). In conclusion, although 24h TT was not affected, the increase in feed intake with PS and the increase and earlier night feeder visits as dietary PS increased indicate a rise in hunger level. Therefore, the hypothesis is rejected. Further, the data suggest that changes in GLP-1 and PYY at physiological levels might not promote satiety in laying hens.

Key Words: slowly digested starch, rapidly digested starch, ileal brake, behavior, satiety

Student Competition: Management and Production I

30 The effects of stocking density on turkey tom core body temperature and litter quality to 16 weeks of age. K. Beaulac*[1], K. Sakamoto[2], T. Crowe[1], and K. Schwean-Lardner[1], *[1]University of Saskatchewan, Saskatoon, SK, Canada, [2]University of São Paulo, Piracicaba, São Paulo, Brazil.*

This study was part of a larger study evaluating the effects of stocking density (SD) (16 wk predicted density of 30, 40, 50, or 60 kg/m^2) on turkey tom performance, health, and well-being. This section evaluated the effects of SD on core body temperature (CBT) and litter quality, while aiming for similar air quality across treatments by altering ventilation rates (NH_3 and CO_2 as indicators). A total of 1,434 Nicholas Select toms were housed in a completely randomized design using 8 independent environmentally controlled rooms (6.71 m × 10.06 m; 2 room replicates per treatment). Feeder and drinker space were allocated on a per bird basis and room temperature and RH were monitored hourly during the trial (iButton Hygrochron). Turkey CBT was evaluated for 9 h in wk 16 (3 birds per replicate) using temperature data loggers administered orally, and recorded crop/gizzard temperature hourly (iButton Thermochron). Two temperature transponders (IPTT-300) measuring surface body temperature were injected subcutaneously beneath the wing and the mid-section of the left breast in each bird and scanned hourly. Carbon dioxide (3 times weekly via hand-held CO_2 meter) and NH_3 (biweekly via Dräger Tubes) were monitored in each room and ventilation was adjusted for differences greater than 20% or 5 ppm, respectively. Litter quality, including moisture and temperature were evaluated from 12 to 16 wk. Litter temperatures were taken 3 times weekly at 4 equally spaced points down the center of the room (18 mm and 36 mm below the surface). Litter samples were taken weekly from 3 locations (front, middle, back) at equal distances (1.0 m) from the drinker line for moisture analysis. Data were analyzed using regression analyses (Proc Reg and Proc RSReg of SAS 9.4) with SD as the independent variable. Significant differences were observed when $P \leq 0.05$. Core/surface body temperature (iButton and wing transponder) decreased linearly as SD increased ($P = 0.03$ and $P < 0.01$, respectively). Overall litter temperature (wk 12–16) at 18 mm and 36 mm responded quadratically to increasing SD ($P < 0.01$ and $P < 0.01$) with the highest temperature observed at 60 kg/m^2. Overall litter moisture (wk 12–16) responded quadratically (37.0, 41.2, 38.5, and 37.4% for 30, 40, 50, and 60 kg/m^2, respectively) as SD increased ($P = 0.02$). Air temperature and humidity (wk 12–16) were not affected by SD. This data demonstrates that increasing ventilation can be used to balance air quality (NH_3 and CO_2), humidity, and temperature as SD increases. In turn, other parameters may be altered such as CBT, litter moisture, and litter temperature, possibly due to cold weather or changes in bird activity.

Key Words: environment, litter moisture, litter temperature, ammonia, carbon dioxide

31 Effect of house type and strain on hen-day egg production, egg quality and tibial bone breaking strength in commercial egg-laying hens in the late phase of production. M. Fulton*, A. Brown, C. McDaniel, and P. Adhikari, *Mississippi State University, Mississippi State, MS.*

The aim of this study was to investigate effects of conventional cage (CC) and enriched colony cage (EC) systems over hen age on both external and internal egg quality parameters, egg production, and tibia breaking strength in 2 commercial laying hen strains, Hyline Brown (HB) and White Leghorns (WL). Hen-day egg production (HDEP) and egg quality data were collected from 53 to 74 weeks of age. Hens were provided ad libitum commercial layer ration containing 2,760 kcal ME/kg and 16% CP. Four groups of hens were assigned as WL in CC (120), HB in CC (120), WL in EC (355) and HB in EC (311). HDEP was calculated every week whereas egg quality was measured at 4-week intervals. A total of 120 eggs were collected to analyze egg quality [egg weight (EW), yolk weight (YW), Haugh unit (HU), albumen weight (AW), shell thickness (ST), shell weight (SW), specific gravity, percentage shell (S%), AW%, and Y%]. At 53 and 74 weeks of age, 6 hens per group were euthanized and their right tibia was collected to measure tibia length (cm), weight (gm) and breaking strength (kg force). Data were analyzed using a 2 × 2 factorial arrangement (house type × strain) in a split-plot in time design with significance set at $P < 0.05$. A 2-way interaction between house type and the strain was significant for HDEP ($P < 0.05$). Both strains of hens in CC had better egg production at 60, 61, 63, 65, 66, 67, 70 and 74 weeks of age (all $P < 0.05$). At 55, 56, 57, 58, 59, 64, 71 and 72 weeks of age, WL had significantly better egg production compared with HB (all $P < 0.05$). Egg weight and AW in CC eggs were higher than EC eggs and increased as hens aged (both $P < 0.05$). For YW, SW, and S%, there were house type, strain, and age effects (all $P < 0.05$). Specific gravity lowered significantly, as hens aged ($P < 0.05$). Tibia length did not differ significantly between hen house type and hen strain. Tibia weight was significantly higher in HB hens in both house types and ages ($P < 0.05$). This study concluded that the egg production and egg size in hens housed in the CC system were greater than that of hens in the EC system. WL hens housed in CC had better production than the HB hens housed in either type. Tibia breaking strength did not differ between the house type or hen strain. An understanding of these different factors is important before moving to an alternative housing system.

Key Words: conventional cage, enriched colony cage, laying hen, egg quality, egg production

32 Early molting of layers: Impact on egg shape and shell quality. A. Miller*[1], D. Jones[2], C. Robison[3], P. Regmi[4], K. Eberle-Krish[5], and D. Karcher[4], *[1]University of Georgia, Athens, GA, [2]US National Poultry Research Center, USDA ARS, Athens, GA, [3]Michigan State University, East Lansing, MI, [4]Purdue University, West Lafayette, IN, [5]North Carolina State University, Raleigh, NC.*

When quarantine zones are established in response to poultry disease outbreaks, such as Highly Pathogenic Avian Influenza (HPAI), flocks within in the zone may not be moved. Pullet rearing facilities in particular can be greatly impacted. The current study was conducted to explore options for holding pullets for an extended period in the rearing system during such a quarantine period. A flock of W-36 pullets were reared in a cage-free housing system from 0 – 16 wks of age. At 17 wks, pullets were assigned to a treatment: Control (CN) – moved into aviary laying facility following commercial practices; Enriched (EN) – stimulated to lay eggs in the pullet facility, perches and nest boxes provided; Molted (MT) – stimulated to lay eggs in the pullet facility, at 10% production molted; and Non-molted (NM) – stimulated to lay eggs in the pullet facility, no enrichments provided. At 25 wks of age, all hens were housed in commercial aviary housing and egg shape and shell quality characteristics were monitored weekly through 29 wks of age. Each week, 36 eggs/treatment were assessed for: weight, length, width, shape index,

volume of shell, specific volume, percent length at maximum width, shell strength, shell elasticity, and shell thickness. A generalized linear model was utilized for statistical analysis. Average egg weight across all treatments increased 2.24 g over 4 wks ($P < 0.01$). While egg length increased ($P < 0.0001$) with hen age, the overall change was <1 mm and not easily perceivable by the human eye. The percent egg length at maximum width decreased with hen age ($P < 0.001$) gradually decreasing 1% of the total egg length between 25 and 29 wks of age. Hen age and treatment interactions ($P < 0.05$) existed for egg width, volume of shell, specific volume, and shell thickness. Generally, CN and MT treatments produced eggs with similar, high quality shells. Early molting of pullets does not appear to negatively impact egg shape or shell quality in commercial cage-free aviary housing systems.

Key Words: pullet, laying hen, molt, egg shape, egg quality

33 Evaluating alternative litter amendments in an attempt to improve broiler litter quality. A. Alqhtani*[2], T. Tabler[1], K. Wamsley[1], P. Adhikari[2], C. McDaniel[5], and A. Kiess[4], *[1]Mississippi State University, Starkville, MS, [2]Mississippi State University, Mississippi State, MS.*

Reducing bacterial populations on broiler farms is critical to maintaining bird health as well as improving litter quality. Broiler litter harbors bacteria, and depending on the species present, ammonia production can become a major issue. Currently, ammonia is controlled by the use of litter amendments such as sodium bisulfate. However, current litter amendments are effective for only a limited amount of time. In vitro studies have indicated that a copper compound (CC) as well as propionic acid (PA) are bactericidal and therefore may have potential to become additional litter amendments. Therefore, the objective of this study was to determine if these new compounds could improve the quality of used broiler litter. For this study, 60 pens ($12ft^2$) filled with 4 inches of used broiler litter were distributed among 6 treatments (trt) (6 trts × 10 pens/trt = 60 pens). Treatments consisted of a control (no amendment), sodium bisulfate, low CC, high CC, low PA, and high PA. On d 0, 3, 7, 10, and 14, ammonia volatilization was assessed and pooled litter samples were analyzed to determine pH, moisture content, as well as total aerobic and *Clostridia* bacterial counts. A solid sided broiler house was divided into blocks by linear location, and data were analyzed using a randomized complete block design with a split plot in time. Differences were separated using Fisher's Protected LSD ($P \leq 0.05$). The results revealed a treatment by day interaction ($P < 0.0001$) for pH, where sodium bisulfate had a lower litter pH on d 7 when compared with all other treatments. However, no differences were detected for any of the other litter quality variables analyzed. The findings for sodium bisulfate were expected because previous research has demonstrated it lowers litter pH. However, the fact that the CC and PA did not reduce bacterial counts was surprising, because the results of an in vitro study indicated they were both bactericidal. Perhaps in the current study the actual concentration of the compounds used were too low. For this study, the same concentrations of CC and PA found to be bactericidal in our in vitro study were applied to the litter, which was significantly lower than the concentration of sodium bisulfate used. If the organic matter within the used litter bound the compounds and thereby further reduced the effective concentration, it is likely that the compounds were no longer bactericidal. Therefore, future research needs to determine if an appropriate bactericidal concentration of the CC and PA can be achieved within litter and ultimately whether these amendments can improve litter quality.

Key Words: litter quality, copper compound, propionic acid, pathogenic bacteria

34 Evaluation of the accuracy and durability of a commercially available ammonia sensor in a commercial broiler house over multiple flocks. C. Mou*, M. Czarick, and B. Fairchild, *University of Georgia, Athens, GA.*

Atmospheric ammonia (NH_3) at levels beyond 25 ppm can suppress broiler performance via reduction in weight, feed conversion, and carcass yield. Prolonged exposures can inflict respiratory damage, increasing a bird's susceptibility to disease. Therefore, it is essential for there to be the ability to accurately and continuously measure NH_3 within a poultry house environment. While accurately measuring NH_3 in a poultry house is of considerable importance, there is yet to be a commercially viable sensor available to producers that can accurately measure NH_3 continuously in an environment where NH_3 is persistent. Recently, a new electrochemical NH3 sensor, the DOL 53, has been introduced to the market which has been specifically designed to measure NH_3 in livestock facilities. Though testing has been conducted in laboratory settings as well as in houses with relatively low NH_3 concentrations, the sensor has yet to be tested under more challenging US conditions where NH_3 concentrations are higher due to the use of built-up litter. To evaluate the accuracy and life of the DOL sensor, a field study is being conducted in a 12 × 125 m totally-enclosed commercial broiler house on a farm in North Georgia. The DOL sensor is being compared with Chillgard photoacoustic meters which have a stated accuracy of ± 1 ppm. The study utilized a minimum of 2 photoacoustic units and up to 5 DOL sensors. Measurements were made and recorded every minute at the same location over the course of multiple flocks. Periodic calibration checks were conducted on both systems with certified NH_3 (45 and 81 ppm). The first 2 trials tested a single DOL unit. The data from both trials indicated that at NH_3 levels lower than 15 ppm there was high agreement between the DOL 53 and Chillgard. However, at NH_3 concentrations above 20 ppm, the DOL 53 reported lower than the Chillgard. Before the third trial, DOL units were sent back to the manufacturer for testing and analysis where a quality control issue was discovered and addressed. For the third and fourth trials, 5 new DOL sensors were tested. A linear regression analysis indicated a high level of agreement between the DOL units and Chillgard ($R^2 = 0.99$) with little variability between the 5 DOL units. This ongoing field study tentatively shows the DOL 53 having the potential in accurately measuring NH_3 concentrations in a poultry house on a continuous basis. The sensor was within 3 ppm of the Chillgard 67% of the time and within 5 ppm 97% of the time. Testing will continue to evaluate long-term accuracy of the sensor.

Key Words: ammonia, air quality, house management

35 Effects of carbon dioxide on turkey poult performance and behavior. Y. Xiong*[1], M. Candido[2], R. Gates[1], I. Tinôco[2], and K. Koelkebeck[2], *[1]University of Illinois at Urbana Champaign, Urbana, IL, [2]Federal University of Viçosa, Viçosa, MG, Brazil.*

Appropriate ventilation of poultry facilities is critical for achieving optimum performance. Ventilation promotes good air exchange to remove harmful gases, excessive heat, moisture, and particulate matter. In a turkey brooder barn, CO_2 may be present at higher levels during winter due to reduced ventilation rates to maintain warm indoor temperatures. This higher CO_2 may negatively affect turkey poult performance. Therefore, the objective of this study was to evaluate the effects of subjecting tom turkey poults (commercial Large White Hybrid Converters) to different constant levels of atmospheric carbon dioxide (CO_2) on their growth performance and behavior. In 3 consecutive replicate trials, a total of 552 poults were weighed post-hatch and randomly placed in 3 environmental control chambers, with 60 (Trial 1) and 62 (Trials 2 and 3) poults housed per chamber. They were reared with standard

temperature and humidity levels for 3 weeks. The poults were exposed to 3 different fixed CO_2 concentrations of 2,000, 4,000, and 6,000 ppm that were kept constant throughout each trial. Following each trial, the CO_2 treatments were switched and assigned to a different chamber to expose each treatment to each chamber. At the end of each trial, all poults were sent to a local turkey producer to finish growout. For each trial, individual initial and weekly poult body weight, and weekly group feed intake were measured. Poult mortality and behavioral movement were also recorded. Variables were tested for difference using one-way ANOVA, Tukey mean separation, and linear contrast. Results of the analysis indicated that wk 3 and cumulative body weight gain of poults housed at 2,000 ppm CO_2 was greater ($P < 0.05$) than those exposed to 4,000 and 6,000 ppm CO_2. Feed intake and feed conversion were unaffected by different CO_2 concentrations. No significant difference in poult mortality was found between treatments. In addition, no effect of CO_2 treatments was evident in the incidence of spontaneous turkey cardiomyopathy for turkeys processed at 19 wk of age. Poults housed at the 2,000 ppm CO_2 level demonstrated reduced activity and movement indices compared with those exposed to the 2 higher CO_2 concentrations.

Key Words: air quality, behavior activity, carbon dioxide, poultry, turkey poult

36 Effects of 2-nitropropanol and 2-nitroethanol on growth performance, apparent digestibility, intestinal lesion scores, and immune gene expression in *Eimeria*-challenged broilers. P.-Y. Teng*, A. Fuller, and W. Kim, *University of Georgia, Athens, GA*.

Coccidiosis is a disease infected by *Eimeria* spp. which causes economic loss worldwide in the poultry industry. Application of anticoccidial drugs is the conventional means for preventing coccidiosis, but emergence of drug-resistant strains underlies the need for developing alternative strategies for replacement. We conducted an experiment to study the effects of 2-Nitropropanol (NP) and 2-Nitroethanol (NE) on growth performance, apparent digestibility and intestinal lesion scores in 14-d-old Cobb 500 broilers challenged with *Eimeria* spp. A total of 144 12-d-old male Cobb 500 broilers were randomly allocated into 24 battery cages with 6 birds in each cage, and divided to 6 treatments, including non-challenged control, challenged control, 100 ppm NP, 200 ppm NP, 100 ppm NE, and 200 ppm NE. Broilers were fed experimental diets from d 12, and all birds except the unchallenged control group were challenged with *Eimeria maxima* (100,000 oocysts/bird), *Eimeria tenella* (100,000 oocysts/bird), and *Eimeria acervulina* (250,000 oocysts/bird) on d 14. On d 20, growth performance and intestinal lesion scores were evaluated. Spleen, liver and cecal tonsils were collected from 2 birds per pen for later determination of immune gene expression. Data were analyzed as an ANOVA, PROC GLM in SAS (9.4) and Duncan test to separate means with significance levels at $P \leq 0.05$. The results showed treatments with *Eimeria* spp. challenge significantly reduced body weight and feed intake, increased intestinal lesion scores and decreased apparent digestibility compared with non-challenged control group. Birds fed 200 ppm of NP had poorer growth performance than both control groups, but significantly reduced cecal lesion scores (1.91 vs. 1.36) compared with challenged control group. Additionally, supplementation of NP significantly improved AMEn and apparent protein digestibility compared with the challenged control. Interleukin-6 (IL-6) and Interleukin-10 (IL-10) were significantly upregulated in the liver after *Eimeria* spp. challenge. NP supplementation at 100 ppm and both levels of NE showed a significant immune gene expression response in the liver with reduction of IL-6 and IL-10 mRNA expression. Overall, supplementation of NP and NE may induce protective outcomes in gut health and immunity of broiler chickens against *Eimeria* spp infection.

Key Words: coccidiosis, nitrocompound, interleukin, apparent digestibility, lesion score

37 The effect of heat stress on the viability of *Eimeria tenella* in vitro. G. Schneiders*, A. Fuller, J. Nelson, R. Rekaya, and S. Aggrey, *University of Georgia, Athens, GA*.

Eimeria parasite infection is limiting to the intestine and characterized by bloody or watery diarrhea, loss of weight gain, poor feed conversion and moderate to high mortality. Heat stress (HS) is among the major environmental stressors in poultry predisposing broiler chickens to immunosuppression and rendering them susceptible to diseases. There are some suggestions that HS reduces *Eimeria* oocyst output in chickens, however, the relationship between HS and coccidiosis is not elucidated. The objective of this study was to investigate the effect of temperature on viability of *E. tenella in vitro*. We designed an experiment to assess the effect of temperature and duration of exposure to HS on viability of *E. tenella* sporozoites. Tubes containing 10^6 sporozoites in PBS were incubated at 35, 40, 45 and 50°C. The duration of incubation was 0, 60, 120, 180, 240 min. Refrigerated live and lasalocid-dead samples were used as controls. Post incubation samples were refrigerated and stained with fluorescein diacetate (FDA) and propidium iodide (PI) overnight. Viability was accessed by flow cytometry with 20,000 events acquired per sample. Sporozoite counts and morphology were accessed by optical microscopy. Data were analyzed using 2-way ANOVA. From the flow cytometric data, *E. tenella* was affected by temperature. Incubation at 50°C induced a significant drop in sporozoite viability marked by increase in PI and decrease in FDA fluorescence emissions. Incubating at 50°C for 60 reduced viability to 81%, however, at 120 min post-incubation, viability was only 3%. Conversely, sporozoites incubated at 35, 40 and 45°C did not show any considerable changes in viability, remaining between 93.8 and 95.6% throughout the process. Additionally, incubation at 50°C affected sporozoite counts and shape. Since HS stress has been reported to reduce oocyte counts in *Eimeria* infected chickens, we can therefore conclude that, under HS, the viability of *Eimeria* sporozoites is significantly reduced thereby affecting its ability to recycle.

Key Words: *Eimeria*, coccidiosis, viability, heat stress, in vitro

38 Oral antibody to interleukin 10 receptor 2, but not interleukin 10 receptor 1, as an effective *Eimeria* spp. immunotherapy in broiler chickens. M. Arendt*, L. Knoll, and M. Cook, *University of Wisconsin-Madison, Madison, WI.*

Coccidiosis is a major gastrointestinal disease caused by several *Eimeria* species in floor raised chickens. Feeding an antibody to Interleukin 10 (aIL-10) ameliorates the negative symptoms of coccidiosis in broilers, i.e., lack of weight gain, decreased feed conversion and mortality. IL-10 is an anti-inflammatory cytokine, which acts as the stand down signal in immune homeostasis. IL-10 signals by forming a ligand-receptor complex with IL-10 Receptor 1 (IL-10 R1) and IL-10 Receptor 2 (IL-10 R2). IL-10 R1 is a high affinity receptor, while IL-10 R2 is a low affinity receptor; both are required for downstream signaling of IL-10. In this study, we hypothesize oral antibodies to the IL-10 receptors will neutralize the IL-10 signaling pathway equal to or better than aIL-10 to act as an oral anti-coccidiosis immunotherapy. Five sequential feed trials, set up as a 4 (diet antibody) × 2 (coccidia challenge) factorial design, tested oral egg yolk antibodies to a total of 6 IL-10 R1 epitopes and 3 IL-10 R2 epitopes compared with a control antibody diet fed to commercial broiler chicks. Ten pens of 5 chicks/pen/diet antibody/coccidia challenge were housed for 21 d. On d 3 of age, chicks were either infected or not infected with a 10 × dose of a coccidia vaccine containing *E. acervulina*, *E. tenella*, and *E. maxima* (Huvepharma Advent coccidiosis vaccine). Pen feed consumption and mean body weights were assessed weekly (d1, d7, d14, d21); fecal oocyst shedding was assessed on d10. Data were analyzed using a 2-way ANOVA. In studies 1–3, evaluating IL-10 R1 antibodies, no significant interaction on chick weight compared with chicks fed the control antibody was observed. Studies 4 and 5, which evaluated aIL-10 R2 oral antibodies, observed that infected chicks fed aIL-10 R2: epitope 1 overcame the negative effects of coccidia infection and had similar 21-d body weight to uninfected chicks ($p_4 = 0.07$ and $p_5 < 0.05$). No significant difference was seen in oocyst shedding for any of the diet antibodies. We hypothesized that feeding oral antibodies to the IL-10 receptors would result in equivalent anti-coccidial benefits to aIL-10. However, none of the 6 antibodies to IL-10 R1 epitopes yielded any benefits during coccidia infection compared with controls. Two of the 3 oral antibodies to IL-10 R2 showed promising results equivalent

to the aIL-10 immunotherapeutic. This difference may be due to the ability of aIL-10 to neutralize IL-10R2 signaling rather than IL-10 R1 signaling. Further research is needed comparing and combining aIL-10 R2 and aIL-10 to improve current immunotherapy for coccidiosis.

Key Words: *Eimeria*, coccidia, interleukin-10, broiler

39 The I See Inside methodology in the survey of histopathological alterations induced by Eimeria infection on gut and liver of broilers and evaluation of the disease impacts over the performance. I. Soares*, B. Belote, A. Kraieski, R. M. Hayashi, A. T. Silva, and E. Santin, *Federal University of Paraná, São José dos Pinhais, Brazil.*

The I See Inside (ISI) methodology translates the severity of lesions into numeric data and it was applied to evaluate the damages of coccidiosis over the gastrointestinal tract of broilers. A total of 132 broilers (from 1 to 28 d) were divided into the groups: negative control (NC) and 10X *Eimeria* vaccine challenge at d 1 (CH), with 6 replicates of 11 birds each. Weekly, feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were evaluated and samples of liver, duodenum, jejunum and ileum were collected for histology. In the ISI histologic evaluation, there is an impact factor (IF) that ranges from 1 to 3 and is defined for each tissue alteration according to the reduction of the organ functionality. To the extent of each lesion (intensity or frequency) compared with non-affected organ, there is a score (S) that ranges from 0 to 3: score 0 (absence), score 1 (<25% of the area), score 2 (from 25 to 50% of the area), and score 3 (>50% of the area). To obtain a final ISI value, the IF of each alteration is multiplied by the respective score number and the results of all alterations are summed according with the formula ISI = Σ(IF*S), where IF = impact factor and S = score. The obtained data were submitted to one-way ANOVA and Tukey's test ($P < 0.05$). The CH group showed lower BWG (g) ($P < 0.05$) at 1–7d (167.15NC * 134.50CH) and 7–14d (356.26NC * 253.67CH), lower FI (g) ($P < 0.05$) at 7–14 (527.87NC * 448.87CH) and 14–21d (837.57NC * 729.02CH) and worse FCR ($P < 0.05$) at 7–14d (1.48NC * 1.79CH). The CH group presented higher ISI final score ($P < 0.05$) in duodenum at 28d (12.60NC * 16.62CH), in jejunum at 21d (10.82NC * 16.56CH), in ileum at 7 d (10.30NC * 12.74CH), 14 d (8.94NC * 11.86CH) and 21d (7.40NC * 11.46CH) and in the liver at 21 d (3.62NC * 6.1CH) and 28d (3.57NC * 5.65CH). The higher intestinal ISI scores in CH group was associated to higher lamina propria immune cells infiltration in duodenum, jejunum and ileum; increased lamina propria thickness, epithelial immune cells infiltration, increased epithelium thickness, proliferation of enterocytes and presence of oocysts in jejunum and ileum and congestion in duodenum and jejunum. In the liver, CH group presented higher ISI score due to cell vacuolation. The results demonstrated the effectivity of ISI methodology in the survey of coccidiosis lesions and in the translation of these alterations into numbers. All the affected parameters were associated to inflammation, which negatively impacts the performance of broilers, and were not evaluated in other morphometric analysis as villus and crypt measure.

Key Words: coccidiosis, gut health, histopathology, intestinal health index, performance

40 Characterization of myogenic stem cell populations in broiler chickens affected with the wooden breast myopathy. T. Ferreira*[1], L. Kindlein[1], K. Meloche[2], S. Vieira[1], V. Nascimento[1], and J. Starkey[2], *[1]UFRGS, POA, Brazil, [2]Auburn University, Auburn, AL.*

Myogenic stem cells (MSC), also called satellite cells, play a critical role in post-hatch muscle growth and regeneration. Activation of regeneration pathways to repair damaged muscle fibers requires both the proliferation and differentiation of different MSC populations as well as the function of resident phagocytic cells such as anti-inflammatory and pro-inflammatory macrophages. The Wooden Breast (WB) phenotype in broilers is characterized by myofiber degeneration and connective tissue infiltration. Our previous work indicates that in broilers severely affected with the WB myopathy, the resident MSC populations expressing the myogenic regulatory transcription factors, Myf-5 and Pax7, are larger and more proliferative compared with those from unaffected broilers. To further characterize the cellular and molecular changes in skeletal muscle that occur as a result in increased severity of WB, a total of 48 Yield Plus × Ross 708 male broiler chicks were euthanized at 25 and 43 d of age (n = 24 per age), the pectoralis major muscles of each bird were visually scored for WB on a 3-point scale (0 = none; 1 = mild, if palpable hardness was present in less than half the total fillet surface area, and 2 = severe, if hardness was present in more than half of the fillet), and samples (n = 8 per score) were collected from the anteroventral area of the fillet and processed for cryohistology. Serial cross-sections (5-µm thick) were cut from each sample and subjected to immunofluorescence staining, fluorescence microscopy, digital imaging and analysis to determine the proportion of type-1 collagen per field as well as densities of macrophages and MyoD+, Myf-5+, and Pax7+ MSC populations on a per mm^2basis. Data were analyzed using the GLIMMIX and MIXED procedures of PC SAS with WB score serving as the fixed effect and bird as the experimental unit. In both 25 and 43-d-old broilers, the proportion of type-1 collagen per field ($P < 0.05$) as well as macrophages per mm^2($P < 0.05$) were greater in fillets exhibiting severe WB compared with normal fillets. At d 43, broilers with severe WB had larger populations of MyoD+, Myf-5+:MyoD+, and Myf-5+: MyoD+: Pax7+ MSC compared with unaffected broilers ($P < 0.05$). The proportion of Pax7+ MSC as a proportion of total cells was also increased as WB severity increased in 43-d-old broilers ($P < 0.05$). Overall, our results demonstrate that there are significant changes in the resident macrophage and MSC populations as well as the type-1 collagen content in the breast fillets of broilers affected with WB. Further investigation will be required to determine how these changes are involved in the dysregulation of muscle growth and maintenance pathways in broilers affected by WB.

Key Words: broiler chicken, pectoralis major muscle, satellite cell, wooden breast, myogenic stem cell

41 Dose titration of dietary dexamethasone and its effects on mucosal permeability in chickens. A. Duff*[1], K. Chasser[1], W. Briggs[1], K. Wilson[1], G. Tellez-Isaias[2], B. Hargis[2], and L. Bielke[1], *[1]The Ohio State University, Wooster, OH, [2]University of Arkansas, Fayetteville, AR.*

Intestinal barrier function is an essential component of the overall health and production of poultry, and enteric inflammation can increase tight junction permeability, thus predisposing broilers to disease. Effective models capable of evaluating this component of gut health are critical for development of strategies to promote efficient growth performance and flock health. Inclusion of dietary dexamethasone (Dex) ranging from 0.57ppm to 5.13ppm in the feed have previously been shown to induce leaky gut, but are not ideal for long-term studies. The objective of the following experiments were to evaluate lower inclusion rates of Dex in a similar leaky gut model, measured by Fluorescein isothiocyanate-dextran (FITC-d) in serum. Treatments in Exp 1 and

Exp 2 included a control diet or 3 Dex diets with variable inclusion rates: Low, 0.1425ppm; Medium, 0.285ppm; or High, 0.57ppm. Feed treatment was administered from d7 – 14. In Exp 1, body weight (BW) was measured on d7 and 14, plus serum FITC-d on d14. All parameters were evaluated using an ANOVA model and Student's *t*-test for means of separation. Body weight gain (BWG) was affected in a dose-dependent manner, with Control at 217.2 ± 5.4g which was significantly higher ($P < 0.05$) than all treatment groups, in which Low gained 173.5 ± 5.7g, Medium gained 140.7 ± 4.9g, and High gained 123.2 ± 5.5g. Serum FITC-d was highest in Medium, and was the only group significantly higher than Control. Exp 2 was similar to Exp 1, but continued to d28 with weekly BW and serum FITC-d on d14 and 28. Day 7–14 BWG was affected in a dose-dependent manner, with Control at 245.9 ± 6.7g which was significantly higher ($P < 0.05$) than all treatment groups, in which Low gained 187.4 ± 3.4g, Medium gained 148.5 ± 3.3g, and High gained 116.5 ± 2.5g. At d14, Low had significantly higher serum FITC-d levels than Control. However, by d28, all groups had higher serum FITC-d levels while BWG also continued to be lower than Control. This indicated that dietary Dex could be decreased below 0.57ppm and retain its leaky gut effects with potentially fewer effects on overall health and welfare of the chickens, which makes these doses more suitable for full grow-out experiments.

Key Words: dexamethasone, mucosal permeability, FITC-d, inflammation, gut health

42 Comparison of chicken blood gas and chemistry parameters between the portable i-STAT1 clinical analyzer and serum biochemistry panel using Hy-Line commercial white-egg laying hens. Z. Sauer*[1], K. Taylor[2], A. Wolc[2], A. Viall[2], N. O'Sullivan[2], J. Fulton[2], I. Rubinoff[2], T. Schaal[2], and Y. Sato[1], *[1]Iowa State University CVM, Ames, IA, [2]Hy-Line International, Dallas Center, IA.*

This study aims to compare the results of the portable Abbott i-STAT1 analyzer and the Abaxis VetScan VS2 for the glucose (Glu mg/dl), ionized calcium (iCa mmol/L), (Na mmol/L) and potassium (K mmol/L) values. The i-STAT1 clinical analyzer has become an increasingly popular tool in clinical production animal medicine due to its ability to report pen-side results in a cost effective and timely manner when compared with standard benchtop serum biochemistry blood gas and chemistry analysis. Additionally, reference interval variability can be witnessed between commercial types and varieties of chickens making accurate inferences on blood gas and chemistry data difficult for clinicians and field veterinarians without such intervals established. Blood gas and chemistry reference intervals are being established for the 3 genetically distinct commercial varieties of Hy-Line white-egg laying hens used in this study (Hy-Line W-36, Hy-Line W-80 and Hy-Line W-80+) utilizing the i-STAT1 analyzer. The laying hens representing each line were randomly chosen and were clinically healthy at the time of blood collection. Thirty-one blood samples were obtained in the production house from the brachial vein and concurrently analyzed by the i-STAT1 portable device. A subset of these blood samples were reserved, and serum was collected from these samples. The serum samples were then analyzed via VetScan VS2, a benchtop serum clinical biochemistry analyzer, using VetScan Avian/Reptilian Profile Plus reagent rotors. Statistical analysis was performed with ANOVA and Tukey's Studentized Range test for the data included in this study to test for statistical differences in each of the parameters between the 2 methods. Results suggest significant differences in blood gas and chemistry values between the 3 commercial varieties (W-36, W-80, W-80+). Preliminary results indicate more accurate analysis of ionized and total calcium values using the i-STAT compared with the serum biochemistry panel, although additional information not provided by i-STAT (e.g., aspartate aminotransferase, uric acid) are available through the panel. This comparison elucidates the importance of clinical analyzer validations when applying different strategies of diagnostic medicine in poultry medicine. This study also demonstrates the importance of reference interval establishment for genetically distinct chicken varieties when incorporating portable i-STAT1 technology as opposed to using already established reference values for *Gallus gallus* from standard biochemistry panels.

Key Words: blood gas, blood chemistry, laying hen, white egg layer, i-STAT

43 Development of a prototype CD40-targeted peptide vaccine against IBDV. M. Alabdali*[1], C. Vuong[1], W.-K. Chou[1], N. Al Haj Ali[1], M. Kogut[2], and L. Berghman[1], *[1]Texas A&M, College Station, TX, [2]USDA-ARS, College Station, TX.*

IBDV is a major poultry viral disease on a global scale due to its severe immunosuppressive effects in 3–6 week-old chickens, and direct losses due to high mortality (60–90%) in the very virulent form of the disease. The currently used vaccines against IBDV are live attenuated viruses because subunit vaccines typically do not confer protection. However, live vaccines have drawbacks such as the risk of reverting to virulence and spreading of the virulent pathogen to surrounding areas. In addition, live vaccines require a cold chain and often lack uniform response to vaccination. In the current study, we report the efficacy of CD40-targeted VP2-derived synthetic peptides as an alternative vaccination strategy. A challenge model was developed by off-label administration of IBDV D-78, a live vaccine strain with intermediate virulence currently used for oral application. Challenge was either oral or intra-cloacal at 10x or 20x the recommended oral vaccine dose at d 24 of age. Ten days post-challenge, the different challenge modes significantly ($P < 0.05$) reduced the weight of the bursa of Fabricius (BF) with 32%, 42%, 48% and 58%, respectively, when compared with controls. The peptide-based vaccine consisted of 3 different biotinylated VP2-derived synthetic fragments complexed with a biotinylated agonistic monoclonal antibody against chicken CD40, using streptavidin as the central scaffold. Each bird received 50 µg of the respective peptide complexes s.c. at 14 d of age, *i.e.* 10 d before challenge. The vaccine partially prevented B-cell depletion in the BF as judged by flow cytometric quantitation of percentage Bu-1^+ viable positive cells per 10^6 cells. Percentage live B-cells per 10^6 bursal cells was down to 44.2% of unchallenged controls ($P < 0.05$) in the non-vaccinated group, compared with 79.3% in the vaccinated group. In the circulation, the % of viable B-cells per 10^6 leucocytes in the 20x cloacal challenge group was numerically slightly decreased compared with controls ($P > 0.05$), whereas a significant decrease ($P < 0.05$) was observed in unvaccinated groups. Histopathologically, severe lymphocyte depletion in the bursal follicles and increased thickness of the interfollicular septae was observed in the challenged groups, but not in the vaccinated groups. In conclusion, this CD40-targeted peptide delivery strategy may provide an alternative approach to the currently used live vaccines against IBDV.

Key Words: CD40-targeted vaccine, infectious bursal disease, poultry, VP2 peptides

44 Evaluation of relationship between oocyst shedding, body weight gain, and lesion scores during *Eimeria* infection. K. Chasser*[1], A. Duff[1], K. Wilson[1], W. Briggs[1], J. Latorre[2], J. Barta[3], and L. Bielke[1], *[1]Ohio State University, Columbus, OH, [2]University of Arkansas, Fayetteville, AR, [3]University of Guelph, Guelph, ON, Canada.*

Coccidiosis has been a pervasive disease within the poultry industry, and a variety of test parameters are used to measure the effectiveness of treatment strategies. Each of these parameters provide varying insight into the impact of *Eimeria*, may be subjective, and some are possibly influenced by non-disease related activity. Four experiments were completed that examined test parameters of coccidial infection, including body weight gain (BWG), lesion scores (LS), and oocysts per gram of feces (OPG). Each experiment included at least 2 of these parameters for measuring the impact of coccidial infection of chickens and turkeys. In Exp 1, a challenged control (CC) was measured against 3 anti-coccidial groups (AC 1–3), while in Exp 2–4, non-challenged (NC) and CC were compared via BWG and OPG, plus LS in Exp 1, 3, and 4. In all experiments, an *Eimeria* challenge was orally administered. Exp 1 results lacked statistical differences ($P > 0.05$) in lesion scores between groups, but BWG and peak OPG at 6 d post infection were reflective of each other, with CC significantly ($P < 0.05$) improved over AC3. A low correlation was observed, determined by Pearson correlation coefficient, between BWG and LS in Exp 1 (r = −0.43), which suggested that even with a moderate challenge, BWG and LS alone were not enough to provide a full description of the disease impact. In Exp 2, no statistical differences between treatment groups were noted for BWG across all time periods, but at d17 and 24, increased ($P < 0.05$) OPG were noted. Very low correlations were observed between pen BWG d7–14 and OPG d10 (r = −0.24) or pen BWG d14–21 and OPG d17 (r = 0.04), which suggested OPG was not reflective of BWG. In Exp 3, BWG remained unaffected by infection through the test period, but LS were stronger in CC at 0.90 ± 0.14 compared with NC at 0.45 ± 0.11 at d19 ($P < 0.05$). When d19 LS were compared with d20 OPG, a high negative correlation was observed (r = −0.79), which suggested an inverse relationship between OPG and LS. This was perhaps due to the low infection level, which was not in the linear region of an *Eimeria* dose response curve. Exp 4 resulted in a very high positive correlation between d21 OPG and average d21 LS (r = 0.86), whereas a high negative correlation was observed between d21 OPG and d16–21 BWG (r = −0.69). This reflected expected decreased BWG in relation to increased OPG. However, moderate negative correlation was detected between d16–21 BWG and average d21 LS (r = −0.49). These experiments show that inclusion of OPG as a standard test parameter for *Eimeria* infection studies provides a global description of disease that may not be observed with BWG and LS alone, especially in cases of subclinical or very high infection levels.

Key Words: *Eimeria*, fecal shedding, oocyst, coccidiosis, test parameters

45 Protein source affects the response of cage-housed broilers to coccidiosis challenge. R. Savary*, A. Van Kessel, and H. Classen, *University of Saskatchewan, Saskatoon, SK, Canada.*

The nature and amount of dietary protein are considered risk factors for the development of enteric disease in broiler chickens. The objective of this study was to determine the impact of a variety of protein sources on the response of cage-housed broilers to 30x label dose of coccidial vaccine. Un-medicated wheat-based diets, fed from 1 to 28d, were formulated by replacing 30% of the CP from soybean meal (SBM) with CP from corn distillers dried grains with solubles (CDDGS), corn gluten meal (CGM), canola meal (CM), porcine meal (PM) or fish meal (FM). At 21d birds were gavaged with Coccivac-B52 (Merck Animal Health, approx. 2.15×10^5 sporulated oocysts) or 1 mL of distilled water. Response criteria were evaluated at 28d. The experiment was a RCBD in a 6x2 factorial arrangement with each treatment administered to 10 replicate cages of 5 birds. Analysis of variance was completed in SAS 9.4 with significance set at $P \leq 0.05$. Means are listed as control versus challenge or in the following order unless otherwise indicated: SBM, CDDGS, CGM, CM, PM, FM. Coccidial vaccination challenge induced disease as indicated by reduced performance (body weight gain: 685, 653 g bird^{-1}; gain to feed ratio (G:F): 0.70, 0.68), nutrient digestibility (AME: 3290, 2779 kcal kg^{-1} 90% DM) and excreta moisture (75.20, 70.68% moisture), and increased size and digesta content of the gizzard, small intestine, ceca and colon. Protein source affected many of the same traits, and interactions between challenge and protein source were found for G:F, nutrient digestibility, duodenal and cecal pH, empty ileum weight, small intestine content and excreta moisture level. Challenge reduced G:F for all treatments except CM, but the decrease was largest for the PM- (0.70 vs 0.66) and FM-fed (0.71 vs 0.66) birds. In unchallenged birds, there were no differences in AME (kcal kg^{-1} 90% DM) among protein sources (3244a, 3242a, 3356a, 3208a, 3365a, 3326a). Challenge reduced AME for all protein sources with FM and PM again affected the most (2984b, 2986b, 2863bc, 2793bc, 2349d, 2700c). *Eimeria maxima* shedding was greatest for PM- and lowest for CM-fed birds (3.94 and 1.83 log oocyst per g) with all other protein sources being intermediate. The data demonstrate that a successful coccidiosis challenge was induced. The response to coccidiosis was variable among protein sources, but FM and PM consistently displayed responses which could be interpreted as undesirable (e.g., high oocyst shedding and low AME).

Key Words: corn distillers dried grains with solubles, corn gluten meal, canola meal, porcine meal, fish meal

46 Forskolin and butyrate act synergistically in protecting chickens from necrotic enteritis. Q. Yang*, B. Chen, K. Robinson, and G. Zhang, *Oklahoma State University, Stillwater, OK.*

Modulating the synthesis of endogenous host defense peptides is being explored as a novel antibiotic alternative approach to disease control and prevention. We have previously demonstrated that forskolin (FSK), a natural labdane diterpene and adenylate cyclase signaling agonist present in the Indian Coleus plant (*Coleus forskohlii*), synergizes with butyrate in inducing the expression of multiple chicken host defense peptide genes both in vitro and in vivo. Here, we further tested the hypothesis that FSK and butyrate have a synergistic effect on the prevention of necrotic enteritis in chickens. A total of 72 male Cobb broiler chicks were fed a common non-mediated starter diet for 10 d and randomly assigned to one of 8 dietary treatments with 9 birds/treatment. Broilers were fed non-medicated diet or diet supplemented with microencapsulated sodium butyrate (1 g/kg diet) and FSK-containing *C. forskohlii* extract (5, 10 or 25 mg/kg diet) individually or in combination for 3 d, followed by daily oral challenge with *Clostridium perfringens* by mixing respective diets with an equal volume of overnight bacterial culture for 4 d. On the 5th day after initial challenge, all animals were sacrificed, jejunal lesions were scored, and the jejunal content was collected for *C. perfringens* quantification. The data was analyzed using one-way ANONA and post-hoc Tukey test. Our results indicated that butyrate and FSK synergistically decreased jejunal lesions, with the combination of butyrate and 10 mg/kg FSK giving the best outcome ($P < 0.05$). In addition, this combination resulted in a reduction of *C. perfringens* load in the jejunum ($P = 0.08$). Furthermore, a 3-wk feeding trial was conducted to investigate the effect of the butyrate/FSK combination on growth performance of chickens. A total of 288 newly hatched male Cobb Chicks were randomly divided into 6 groups with 6 replicate cages/group and fed standard non-medicated starter diet or the same experimental diets as used in the necrotic enteritis model. Weekly performance was calculated and analyzed using one-way ANONA and

post-hoc Tukey test. The results showed that weight gain of chickens was not significantly different among treatments ($P > 0.05$). FSK alone or in combination with sodium butyrate significantly decreased feed intake and improved feed efficiency from d 7–14 and d 0–14 ($P < 0.05$). Collectively, these results revealed the potential of feeding butyrate and FSK as alternatives to antibiotics for prevention of necrotic enteritis, with the tendency to improve feed efficiency in healthy chickens.

Key Words: antimicrobial resistance, antibiotic alternatives, host defense peptides, necrotic enteritis, butyrate

47 Disease signs, growth, and egg production of broiler breeders infected with *Histomonas meleagridis*. E. Chadwick*, R. Malheiros, A. Ferrarini, and R. Beckstead, *North Carolina State University, Raleigh, NC.*

Histomonas meleagridis, an anaerobic protozoan, is the cause of blackhead disease. Although high mortality is not normally seen in chickens, cecal and liver damage can occur. This can lead to poor growth as well as animal health concerns. Field observations suggest that blackhead disease in broiler breeders results in delayed onset of lay and decreased egg production. Therefore, to directly link infection of *H. meleagridis* on broiler breeder performance, a study was conducted to measure disease signs, growth and egg production. 736 breeder hens were weighed and placed in pens on day of hatch (92 hens per pen). Birds were subjected to vaccinations based on industry standards. On d 25, 4 pens were infected with *H. meleagridis* via cloacal inoculation (100,000 cells per bird) and 4 pens acted as the control. At 5, 10 and 20 dpi, 5 birds per pen (2 birds per pen on d 20) were euthanized and scored for blackhead disease on a 0–4 scale. Individual body weights were collected on wk 3, 5, 13, 15 and 20 post-placement. During wk 13–15, cloacal swabs were collected from the infected pens to determine if *H. meleagridis* could be recovered via PCR. Egg production was recorded once hens were in lay. Data were analyzed in JMP Pro 13 using the GLM Procedure and means were separated using a student's *t*-test for treatment effects ($P \leq 0.05$). Lesions in the ceca averaged 1.6, 3.2 and 1.8 on d 5,10 and 20 for the infected birds, respectively. The liver showed no lesions on d 5 or 10 and minimal lesions on d 20. Control birds had scores of 0 for the ceca and liver lesions. On wk 13–15, *H. meleagridis* was recovered from the cloacal swabs of infected birds, demonstrating that the birds recovered from the disease but did not eliminate the parasite. No differences in pen weights were observed between treatments. The coefficient of variation for individual bird weights differed significantly between treatments on wk 10 and 20. There was no delay in the onset of egg production and at wk 32, the treatments showed no difference in quantity of eggs produced. Although Infection of broiler breeders with *H. meleagridis* caused variation in body weights, it did not cause mortality, alter the onset of lay or alter the quantity of eggs produced. Therefore, high levels of morbidity and mortality seen in blackhead field outbreaks is not only a result of *H. meleagridis* infection.

Key Words: broiler breeder, *Histomonas meleagridis*, egg production

48 Antibiotic alternative deoxycholic acid alleviates chicken necrotic enteritis. M. Bansal*, M. Abraha, B. Al-Rubaye, A. Almansour, H. Wang, J. Latorre, B. Hargis, and X. Sun, *University of Arkansas, Fayetteville, AR.*

Necrotic enteritis (NE) has reemerged as a prevalent chicken disease with huge losses every year after withdrawing antimicrobial growth promoters. Coccidia and *Clostridium perfringens* are the main pathogens responsible for NE. Very few effective antibiotic alternative is available to prevent or treat NE. Here we hypothesize that microbiota metabolite secondary bile acid deoxycholic acid prevents NE. To examine this hypothesis, broiler chicks in pen housing were randomly allotted to 4 diets supplemented with 0 (basal diet), 0.8, 1.0 or 1.5 g/kg (on top of basal diet) deoxycholic acid (DCA). At 18 d of age, birds were orally infected with *Eimeria maxima* (20,000 sporulated oocysts/bird) to induce coccidiosis. The birds were subsequently infected with 10^9 cfu/bird of *C. perfringens* at d 23 and 24 d of age to initiate NE. Growth performance of body weight gain (BWG), feed intake and feed efficiency were measured at d 18, 23, and 26. The birds were sacrificed at 26 d of age. Ileum tissue and content were collected for *E. maxima* and *C. perfringens* colonization, and histopathology. Histopathology score was based on criteria of villus shortening, immune cells infiltration and crypts hyperplasia. Consistent with previous reports, daily BWG in birds challenged with NE were significant reduced compared with non-infected birds at NE phase of 23–26 d of age (56 vs. 77 g/bird, $P = 0.03$). Notably, DCA (1.0 g/kg) attenuated *E. maxima* and *C. perfringens* induced growth impairment of daily BWG compared with NE control birds at d 23–26 (NE phase, 68.28 vs. 56.01 g/bird, $P = 0.02$), while birds fed diet with DCA at 0.8 and 1.5 g/kg showed trend of improved daily BWG compared with NE control birds. At cellular level, *E. maxima* induced mild intestinal inflammation and subsequent *C. perfringens* infection-induced NE showed inflammation of shortening of villi, crypt hyperplasia, and infiltration of massive immune cells in lamina propria. Remarkably, DCA diets (0.8, 1.0 and 1.5 g/kg) significantly reduced the NE induced intestinal pathology compared with NE birds (ileitis score 6.6, 5.6, 2.8 vs 12.4 respectively). Because DCA reduces *C. perfringens in vitro* growth, the bacterial load was examined in chicken ileal content using real time PCR. *C. perfringens* colonization was increased in NE birds while DCA diets (1.0 and 1.5 g/kg) significantly reduced the pathogen colonization compared with NE control birds (2.6×10^5 and 2.5×10^7 vs 5.0×10^8 cfu/g ileal digesta, respectively). In conclusion, DCA attenuates *C. perfringens* induced growth impairment and NE pathology, possibly through attenuating the pathogen colonization.

Key Words: necrotic enteritis, deoxycholic acid, ileitis score, illeal content, pathogen colonization

49 Effect of dietary zinc sources and necrotic enteritis challenge in broiler chickens. C. Bortoluzzi*[1], B. Lumpkins[2], G. Mathis[2], WD King[3], D. Graugnard[3], K. Dawson[3], and T. Applegate[1], *[1]The University of Georgia, Athens, GA, [2]Southern Poultry Research Inc., Athens, GA, [3]Alltech, Nicholasville, KY.*

Zinc (Zn) is an essential micromineral required for growth of animals, modulation of cytokine production and may modulate intestinal development and/or regeneration during and after enteric disease. The objective of this study was to compare the effects of 2 sources of Zn (inorganic and organic) on the performance, intestinal integrity, and expression of immune-related genes of broiler chickens induced to necrotic enteritis (NE). One-day-old male Cobb 500 broiler chicks were fed dietary treatments in a 3x2 factorial design (8 cages/treat; 8 birds/pen) with 3 dietary formulations (0 or 90 mg/kg supplemental Zn from $ZnSO_4$ or organic Zn (Bioplex Zn, Alltech Inc.)), with or without challenge. On d 14, challenged birds were orally gavaged with ~5,000 *Eimeria maxima* sporulated oocysts, and on d 19- 21 were given a broth culture of *Clostridium perfringens* (10^8 cfu/d). Feed intake (FI), BW gain and feed conversion ratio (FCR) were assessed at d 21 and 28. On d 21, 3 birds from each cage were scored for NE lesions, and intestinal permeability was measured by FITC-dextran (mol wt 4,000) and its passage into the blood; jejunum and cecal tonsils were collected for gene expression analysis. There was no interaction between Zn source

and NE challenge on performance on d 21 and 28. The challenge negatively affected ($P < 0.05$) FI, BW gain, and FCR on d 21 and 28, with no effect of the Zn supplementation. An interaction of Zn source and challenge was observed for lesion score ($P = 0.02$) and mortality ($P = 0.008$), wherein Zn supplementation decreased the degree of NE lesions (from 1.42 to 0.96 on a 0–3 point scale) and mortality due to NE (from 28.1 to 14.1%). There was no interaction between Zn source and challenge on the FITC-d passage from the intestine to the blood, nor effect of the Zn supplementation. In the jejunum, an interaction of Zn source and challenge was observed for the expression of IL-8 ($P = 0.001$) and INF-γ ($P = 0.03$), wherein the NE challenge upregulated their expression, but not in the organic Zn supplemented birds. NE challenge also upregulated the expression of IL-10 ($P = 0.001$) and iNOS ($P < 0.0001$). Additionally, organic Zn supplementation downregulated iNOS vs. ZnSO4 supplemented birds ($P = 0.0003$), and supplemental Zn downregulated TLR-2 ($P = 0.05$), regardless of the source. In the cecal tonsils, NE challenge upregulated the expression of IL-8 ($P = 0.02$), INF- γ ($P = 0.02$), iNOS ($P = 0.0007$), and downregulated the expression of NFK-β ($P = 0.0003$). NE challenge impaired the performance of the chickens. Dietary Zn did not counteract this effect, but decreased NE lesions and mortality due to NE; organic Zn led to lower expression of IL-8 and INF- γ which indicates a lessened inflammatory response.

Key Words: necrotic enteritis, coccidiosis, organic zinc

50 Microbial metabolite deoxycholic acid in drinking water prevents chicken necrotic enteritis. M. Abraha*, M. Bansal, B. Al-Rubaye, A. Almansour, H. Wang, J. Latorre, B. Hargis, and X. Sun, *University of Arkansas, Fayetteville, AR.*

Necrotic enteritis (NE) caused by *Clostridium perfringens* and coccidiosis is one of the most important diseases with billions of dollars loss annually. The withdrawing antimicrobials in poultry industry is the main contributing factor for the increasing incidence of NE. Because dietary medicine delivery is impaired in NE birds with reduced feed intake, the objective of current research was to reduce NE using microbiota metabolic product secondary bile acid deoxycholic acid (DCA) in drinking water. Broiler chicks in floor pens were challenged with *Eimeria maxima* (20,000 oocysts/bird) to induce coccidiosis at d 18. At d 23 and 24, the broilers were infected with *C. perfringens* (10^9 cfu/chicken/day) to induce NE. The birds were weighed and feed intake was recorded at d 18, 23, and 26. DCA was administrated in drinking water at 0.075% from d 16 to 26. At d 26, the broilers chickens were sacrificed. Samples of small intestinal content and tissue were collected and processed for isolating DNA, RNA, and histopathology. Notably, birds infected with *E. maxima* and *C. perfringens* developed mild NE and suffered growth performance reduction of daily body weight (BW) gain compared with noninfected birds (56 vs. 76 g/bird, $P = 0.03$), while DCA in water prevented the BW loss (56 vs. 71 g/bird, $P = 0.07$). Upon examination of the bird histopathology, small intestine of NE birds displayed obvious intestinal inflammation showed as shortening of villi, hyperplasia of crypt, and infiltration of inflammatory cells into intestinal lamina propria. Importantly, DCA alleviated the NE-induced intestinal inflammation (pathological score 12.7 vs. 7.2, $P = 0.04$) compared with NE control birds. At molecular level, DCA reduced inflammatory mediator of *Infγ* mRNA accumulation in the small intestinal tissue by 72% compared with that from NE control birds. Remarkably, DCA in drinking water reduced *C. perfringens* luminal colonization in small intestine compared with that in NE control birds ($5x10^6$ vs. $5x10^8$ cfu/g digesta, $P = 0.04$). In conclusion, microbial metabolic product DCA in drinking water prevents NE-induced BW loss and small intestinal inflammation. These findings pave the path for treating NE using DCA in drinking water.

Key Words: necrotic enteritis, broiler, *Clostridium perfringens*, colonization, deoxycholic acid (DCA) in water

51 Ovulation of the largest preovulatory follicle and follicle recruitment to the preovulatory hierarchy are not temporally linked in the laying hen. K. Ghanem* and A. Johnson, *Pennsylvania State University, University Park, PA.*

In the laying hen it is generally assumed that ovulation of the largest preovulatory (F1) follicle and recruitment of the smallest preovulatory (9–12 mm) follicle are coordinated events. The ability to predict the time of recruitment would inform investigations of follicle recruitment by enabling one to predict, and eventually identify, the most proximal signal(s) that result in follicle recruitment. The objective of this study was to determine if recruitment and ovulation are temporally linked in the laying hen ovary. We hypothesized on a day when ovulation fails to occur, recruitment also fails to occur. Hy-Line W-36 hens, 68–73 weeks of age were divided into 3 groups: no ovulation, first ovulation, and late ovulation. The time and occurrence of oviposition and ovulation were documented by video together with palpation 1 h after oviposition. The ovary was collected during the last hour of photoperiod, and the reproductive tract was examined to verify if ovulation occurred. Ovarian follicles were collected and weighed. A follicle weighing between 300 mg and 900 mg, with a yellow yolk appearance and a diameter of 9–12 mm was considered to have been recruited that day. To compare the rate of yolk uptake in 9–12 mm follicles among the 3 groups, hens were fed gelatin capsules containing Sudan IV dye 6 h before ovary collection. The most recently recruited follicle was collected, weighed, boiled and sectioned. Images of sections were taken using a dissecting microscope and analyzed using ImageJ software. Finally, cell cycle analysis of granulosa cells (GC) from the 9–12 mm follicle was assessed by flow cytometry. Here we report that there was no significant difference in the weight of the 9–12 mm follicle from hens that did not ovulate compared with the control groups (670 ± 80 mg, 560 ± 130 mg, and 650 ± 120 mg for no ovulation (n = 5), first ovulation (n = 3) and late ovulation (n = 4), respectively; $P = 0.8$). Further, dye uptake measurements showed that the 9–12 mm follicle from each group was actively incorporating yolk with no significant difference in the percent of dyed-yolk uptake among the 3 groups (19.02 ± 2.03%, 18.02 ± 3.66%, and 19.83 ± 3.20% for no ovulation, first ovulation, and late ovulation, respectively; $P = 0.9$). Finally, cell cycle analysis indicated active proliferation in GC of 9–12 mm follicle with 21.80 ± 5.79%, 18.08 ± 0.36%, 14.80% of GCs in the S or G2-M phase of mitosis) in no ovulation (n = 3), first ovulation (n = 2), and late ovulation (n = 1), respectively, indicating that 9–12 mm follicles from each group were actively growing. We conclude that follicle recruitment and ovulation of the F1 follicle in the laying hen are not temporally linked.

Key Words: ovary, ovulation, follicle recruitment, prerecruitment, preovulatory

52 Physiological impact of genetic selection for laying persistency. C. Hanlon*, K. Takeshima, and G. Y. Bédécarrats, *University of Guelph, Guelph, ON, Canada.*

In the laying hen industry, current breeding strategies focus on selecting for earlier maturation and extended laying periods thus, indirectly impacting the activity of the hypothalamic-pituitary gonadal axis. The objectives of the study are to determine at what level of the axis changes occurred by comparing 3 strains of layers with various degree of selection for egg production. The Lohmann LSL-lite (L) was used as current commercial strain, the heritage white Leghorn Shaver (S) was used as midpoint selection strain, and the Smokey Joe strain (J) was used as non-selected strain equivalent to a 1960s layer. Chicks were housed in brooding cages until 12 weeks of age (woa), then randomly assigned per strain to colony cages (2 per cage). Photoperiod was set at 10h until photostimulation at 18woa (12h light), increasing 1h/week to 16h (22woa). Body growth was followed from day-old chicks, egg production was recorded and circulating estradiol (E2) profiles were established through repeated blood samples (n = 50 hens/strain) beginning at 12woa until the end of trial (100woa). In addition, hens were sacrificed over time for ovarian measures and egg quality was monitored throughout lay. Data was analyzed using the MIXED procedure in SAS. At 85woa, flock averages throughout the laying cycle were 90.6% (418 eggs), 74.1% (339 eggs), and 47.0% (206 eggs) for L, S and J hens, respectively, confirming that genetic selection was responsible for more than doubling the hen egg output per cycle. From 38woa onwards, production of L was significantly higher than S and J ($P < 0.001$). The initial rise in E2 associated with sexual maturity was observed at 19woa for L and S ($P < 0.001$), while J was delayed to 21woa ($P < 0.0001$). Interestingly, subsequent recurrent increases in average E2 were observed, with 3 additional increases for L (32–36, 46–47, 68–72woa) and 4 for S (26, 32–34, 56–60, 72woa) through 82woa. Average E2 levels were higher in L than S at 18, 46, and 80woa ($P < 0.04$), while S were greater at 54woa ($P = 0.04$). Meanwhile, the ovary was heavier in L than S and J at 25, 45, and 75woa ($P < 0.001$), ages at which average E2 levels were also higher. Individual profiles show variability in E2 profiles within breed, however, cyclical rises in E2 could be observed for L and S hens. Egg weight within breeds was maintained from 60 to 80woa, however, eggs from L were heavier than S at 80woa ($P = 0.0347$). While shell thickness of L eggs was highest at 26 and 40woa then declined, it remained constant for S and J eggs. In conclusion, our results suggest that prolonged lay is associated with cyclical increases in E2, possibly resulting from multiple successive waves of follicular recruitment and continuous stimulation of the reproductive axis.

Key Words: estradiol, laying hen, laying persistency, reproduction, genetic selection

53 Changes in gastrointestinal proteome of chicks after in ovo administration of different bacterial isolates. K. Wilson*[1], W. Briggs[1], A. Duff[2], K. Chasser[1], W. Bottje[3], L. Zhang[1], and L. Bielke[1], *[1]Ohio State University, Columbus, OH, [2]The Ohio State University, Wooster, OH, [3]University of Arkansas, Fayetteville, AR.*

Pioneer colonization of the gastrointestinal tract (GIT) by microbiota is thought to have major influence on tissue development of neonatal animals. Studies have shown that in ovo inoculation of perinatal chicks with saline (S), *Citrobacter* (C), *Klebsiella* (K) or a LAB-probiotic (L) resulted in an altered microbiome composition on day of hatch (DOH; $P < 0.01$). The current study investigated changes in proteins of the GIT at DOH in relation to these microbial inoculations. Embryos were inoculated with S, or approximately 10^2 cfu of C, K or L on embryonic d18. On DOH, the whole-GIT were collected, contents removed and protein extracted for analysis via tandem mass spectrometry (Fusion Mass Spectrometer, LC-MS/MS). Proteins were identified through Sequest with the *Gallus gallus* reference. A total of 493 proteins were identified for differential comparison to S at a significance level of $P \leq 0.10$. Different levels were noted in 108, 39, and 78 proteins in C, K, and L groups, respectively, which were uploaded to Ingenuity Pathway

Analysis to determine associated canonical pathways and biological functions related to these changes. Upstream regulators in the cytokine family upregulated by K, indicated with a z-score ≥ 1.000, included TNF, IFNγ, IL1β, IL6, IL4, OSM, CSF2, CD40L, IL2, and IL13, which suggested an overall pro-inflammatory condition of the GIT. This was in contrast to heavy downregulation, z-score ≤ 1.000, or no change in regulation, to these cytokines in L treated groups, which would indicate a state of decreased inflammatory status within the GIT. This was further illustrated by increased activity (z ≥ 1.000) of 229 molecules associated with cellular assembly, organization, function and maintenance in K infected samples, versus overall downregulation in L treated samples, which could be associated with cellular injury and repair. Along these same lines, K increased (z ≥ 1.000) activity, as opposed to no change in L, in 360 molecules related to migration of immune cells in the GIT. The L treatment exclusively upregulated leukocyte extravasation signaling (z = 1.211, P = 1.21E-09), VEGF signaling (z = 2.334, P = 1.57E-09) and cardiovascular NO signaling (z = 1.890, P = 1.72E-03). While systems and pathways affected by K and L generally contradict each other, the relationship of C was variable. These changes in proteome suggest that pioneer colonizing microbiota can have a strong impact on several pathways associated with immune function and cellular development in the GIT.

Key Words: microbiota, proteome, GIT, canonical pathways

54 Proteomics changes induced by sodium butyrate on chicken enterocytes cell culture model. A. Gupta*[1], A. Upadhyay[1], R. Liyanage[1], N. Rath[2], A. Donoghue[2], and J. Lay Jr.[1], *[1]University of Arkansas, Fayetteville, AR, [2]Poultry Production and Product Safety Research Unit, ARS, USDA, Fayetteville, AR.*

Butyrate is a short-chain fatty acid synthesized by gut microbiota. In the human gut, butyrate regulates epithelial transport, inflammatory pathways, and barrier function. However, a paucity of information exists on the effect of butyrate on chicken gut physiology. The objective of this study was to develop a chicken primary epithelial cell culture model and to study the effect of butyrate on various physiological/biochemical pathways proteins using liquid chromatography tandem-mass spectrometry (LC-MS/MS). Intestinal villi were harvested from day old broiler chicks in Dulbecco's Modified Eagle Medium/ F-12 (DMEM/F-12) followed by sequential digestion (Hyaluronidase/Trypsin) and density gradient centrifugation (Histopaque). Dissociated cells were plated in DMEM containing antibiotics, Fetal Bovine Serum, insulin transferrin selenium and epithelial growth supplement. Cells were cultured (10^5 cells/well) to semi-confluency and treated with sodium butyrate (100, 10, 1ug/ml) for 24 h. The treated cells were lysed and proteins subjected to electrophoresis (4–20% gradient gel for 90 min). Gel segments were reduced/alkylated followed by trypsin digestion. Peptides were evaluated to identify and quantify proteins using LC-MS/MS. Differentially regulated proteins were determined using Scaffold software (Fisher's Exact Test, P < 0.05). A total of 234 proteins were identified. At least 30 proteins were uniquely present in control cells, 11 proteins in butyrate treated cells and over 176 proteins were common in both. Gene ontology revealed that majority of proteins contribute to cellular activity, biological regulation and metabolic function. Butyrate increased the expression of proteins contributing to critical biological functions including cell integrity (VIME, F1NJT4, Q05705) and physiological response (E1BV01, Q7ZT27, B3VHV2). The butyrate treatments reduced the expression of proteins contributing to actin binding (Q02015, E1C2S1, Q90574, ACTC), pro-inflammatory response (MIF), and signal transduction (HS90A, 1433Z, 1433G). Results suggest that butyrate may have impact on cytoskeletal changes, energy metabolism in chicken epithelial cells and also have potential to inhibit histone deacetylase that plays a role in cancer. Protein-protein interaction analysis (STRING) is currently underway to understand the effect of butyrate on major protein groups critical for gut health in poultry.

Key Words: epithelial cell culture, butyrate, intestinal health, proteomics

55 Effect of Noni (*Morinda citrifolia*) on gene expression in breast muscle from broilers exposed to heat stress. G. Damico*, K. Lassiter, J. Flees, E. Greene, S. Dridi, and W. Bottje, *[1]University of Arkansas, Fayetteville, AR.*

Heat stress (HS) is known to adversely affect commercial poultry production. Dried Noni plant (*Morinda citrifolia*) has been shown to ameliorate the negative effects of heat stress; However, the mechanisms responsible are not completely understood. Thus, this study was conducted to determine the effect of chronic HS on gene expression in breast muscle and to assess if the dried Noni plant modulates HS effects in vivo. Broilers (480 males, 1d) were randomly assigned to 12 environmental chambers; with 2 pens per chamber (randomized) with separate feeders and water lines. Chicks (20/pen) were fed a corn-soy based diet (Control) or one containing 2 g dried Noni plant/kg feed (Noni) and brooded under thermoneutral (TN) conditions for 21 d. On d 22, temperature was raised to 35C (HS) in 8 chambers for 3 wk, with the remaining 4 chambers maintained at TN temperature (25 C). After 3 wk of chronic HS, birds (1 per pen randomly selected from each pen), were euthanized by cervical dislocation. A portion of breast muscle was removed, flash frozen in liquid nitrogen before storing at −80 C. RNA was isolated from the muscle tissue and targeted gene expression conducted by quantitative real-time polymerase chain reaction (qRT-PCR). Data were analyzed by 2-way ANOVA with temperature and diet as the main effects. Means were compared by Tukey's multiple comparison and student's t-test and considered significant at P < 0.05. The results indicate that after 3 wk of chronic HS, a main effect of temperature was observed in the upregulation of heat shock protein 70 (HSP70), mechanistic target of rapamycin (mTOR), and peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC1-a) and downregulation of adenine nucleotide translocase (ANT) in HS compared with TN broiler breast muscle. HS broilers provided Noni exhibited a moderate downregulation (P < 0.09) for HSP70, mTOR, PGC1a compared with HS broilers fed the Control diet. Noni-fed broilers exposed to HS exhibited downregulation of ANT and upregulation of avUCP compared with broilers fed Noni that were maintained under TN conditions. The results indicated that HS upregulated HSP70, mTOR, PGC1a and downregulated ANT in broiler breast muscle and that these changes in gene expression may be attenuated in broilers provided dried Noni plant. Studies are currently underway to determine effects of Noni on gene expression in muscle following 2 h of acute heat stress.

Key Words: broiler, heat stress, Noni plant, gene expression

56 Steroidogenic follicular response differences to LH and FSH treatment in low- and high-egg-producing turkey hens. K. Brady*[1], J. Long[2], and T. Porter[1], *[1]University of Maryland, College Park, MD, [2]USDA, Beltsville, MD.*

Low egg production in turkey hens is correlated with decreased ovulation frequency. Ovulation frequency is governed at the ovarian level by the steroid hormones progesterone and estradiol. The avian ovary is comprised of follicles in varying stages of maturation, which serve specialized roles in steroidogenesis. The F1 follicle of the preovulatory hierarchy is the next follicle in line to ovulate. Cells from the granulosa

layer of the F1 follicle (F1G) produce the majority of ovarian progesterone in response to luteinizing hormone (LH). Small white follicles (SWF) are slow growing follicles that have not entered the preovulatory hierarchy and produce the majority of ovarian estradiol in response to follicle stimulating hormone (FSH). We reported previously that mRNA levels for enzymes related to progesterone and estradiol production are differentially expressed within the turkey hen ovary between low egg producing hens (LEPH) and high egg producing hens (HEPH), with HEPH exhibiting higher expression of genes encoding key steroidogenic enzymes when compared with LEPH ($P < 0.05$). In the present study, the main sources of progesterone and estradiol, the F1G and SWF cells, were cultured with LH and FSH, respectively, to determine if cells from HEPH displayed an increased response to LH and/or FSH. F1G cells from LEPH and HEPH (n = 4) were cultured with 0, 1, 10, 100, or 1000 ng of LH, while SWF cells from LEPH and HEPH (n = 4) were cultured with 0, 1, 10, 100, or 1000 ng of FSH. After 5 h incubation, media from the F1G and SWF cell cultures were assayed for progesterone and estradiol, respectively, by radioimmunoassay. Results were analyzed by a 2-way ANOVA using the mixed models procedure of SAS. F1G cells from HEPH produced more progesterone than cells from LEPH at 10, 100, and 1000 ng of LH treatment ($P < 0.05$). SWF cells from HEPH produced more estradiol than cells from LEPH in response to 10, 100, and 1000 ng of FSH ($P < 0.05$). Cells from HEPH displayed a higher sensitivity than cells from LEPH to both LH and FSH treatment in vitro. The increased sensitivity to LH and FSH, coupled with the upregulation of genes that support steroidogenesis, may play a role in the increased egg production exhibited by the HEPH. Further examination of the genetic contribution to differences between LEPH and HEPH at the transcript and hormone response level is needed to identify additional factors contributing to increased egg production in turkey hens.

Key Words: egg production, cell culture, follicle, progesterone, estradiol

57 Cloacal microbiota changes in broiler breeder hens with change in housing and feed intake. L. N. Isaula*, *Texas A&M, Bryan, TX.*

Increasingly indigenous microbiota (primarily bacteria) of chickens is considered to affect feed conversion, weight gain and health. Broiler breeder hens are prone to obesity and are managed with significant feed restrictions FR to improve egg production. Acute release from FR is used to study the effects of increased feed intake on breeder hen metabolism and health; but, possible changes in gut microbiota are unstudied in this model system. To address this gap, 30, 29 week old, Ross 708 hens (87.8 hen-day %) were individually housed and fed to breeder standard (167g of 2750kcal/kg ME diet/hen/day). After 10 d of adaptation period cloacal swabs were collected and 10 hens continued with 167 g feed/day (FR) and 20 hens with334g/hen/day (UR) for 10 experimental days when a second cloacal swab was collected. Cloacal swabs allowed for repeated sampling of individuals and estimate of microbiota likely to be deposited on eggs. Bacterial DNA was extracted and 16S rRNA gene sequenced using an Illumina platform (MrDNA, Shallowater, TX). Bacterial diversity and richness were analyzed using QIIME and the bacteria functionality predicted with PICRUSt. Unrestricted hens gained 386 ± 32 g body weight in 10 d while the FR hens gained 18 ± 27 g during this same interval ($P = 0.001$). Species richness (α – diversity) increased over baseline values by 154% and 146% in FR and UR groups, respectively, (Chao1 values, $P < 0.004$). The β-diversity, indicator of differences in bacterial communities, in the initial and final cloacal swabs were found (unweighted Unifrac distance, ANOSIM $P = 0.003$) for combined FR and UR groups. Energy intake per se had no effect albeit changes were not identical in the 2 groups. In FR hens 6 bacterial genera increased significantly with 5 of the 6 being among the 13 bacterial genera that increased significantly in UR hens . Overall, *Firmicutes* genera decreased in abundance with substitution by *Proteobacteria* genera. PICRUSt estimated bacterial functional capacity showed similar increases in 10 processes related to environmental change in both FR and UR hens. In UR hens an additional 9 processes, primarily related to metabolism, protein and glycan synthesis and signaling increased. Modification in breeder hen cloacal microbiota occurs in response to changes in housing and energy intake. These factors primarily impact community composition and function, respectively.

Key Words: feed restriction, overfed, broiler breeder hen, gut microbiota

58 Effects of early-life stress on embryonic mortality, hatching weight and growth are dependent on stress model, maternal age, and genetic line in laying hens. M. Peixoto*, N. Karrow, T. Widowski, and A. Newman, *University of Guelph, Guelph, ON, Canada.*

Early-life exposure to stressors can shape the phenotype of the offspring resulting in changes that may permanently affect health and wellbeing. This can be modelled indirectly through maternal exposure to stressors (Natural Model), or by direct exposure of the offspring to stress hormone (Pharmacological Model). In this study, both models were utilized to investigate the effects of genetic line and maternal age on offspring embryonic mortality, hatching weight and growth. To form the Parent Stock, fertilized eggs of 4 commercial genetic lines — two brown (B1 and B2) and two white (W1 and W2) — were incubated, hatched and housed identically in 4 flocks of 27 birds (24F: 3M) per strain. Each strain was equally separated into two groups: "Stress", where hens were subjected to a series of acute psychological stressors (e.g. physical restraint) for 8 days prior to egg collection, and "Control", which received routine husbandry. When parents were 32, 52 and 72 weeks of age (woa), fertile eggs from both treatments were collected and additional eggs from Control were injected with corticosterone (10 ng/mL egg content)("CORT"). A "Vehicle" treatment was included to account for effects of egg manipulation. Eggs were incubated, hatched, and offspring (N=1152) were brooded until 17 woa under identical conditions. The effects of stress model, genetic line and maternal age were analyzed as a 2-way ANOVA using the Glimix procedure. Interactions between maternal stressor and genetic line, and maternal stressor and age were tested. Natural stress did not interact with genetic line to affect traits. However, eggs laid by 32 woa hens from Stress showed better hatchability and hatched lighter offspring than ones laid at 72 woa ($P < 0.0269$). The pharmacological model consistently decreased hatchability in all strains and maternal ages. Similarly, CORT decreased progeny body weight at hatch at all maternal ages. Overall, B2 was the most susceptible strain to the pharmacological model, showing the highest levels of embryonic mortality, early embryonic death and lower average daily gain (ADG) to 17 woa. Compared to the Control, CORT also displayed decreased ADG in B1 ($P = 0.0097$) and W2 ($P < 0.0001$), but not W1 ($P = 0.7091$). These results show that early-life stress interacts with strain and maternal age to affect hatchability, hatching weight and growth of the offspring. Moreover, it suggests that the natural stressor model may be useful for quantifying the response of the mother to stressors, whereas the pharmacological model may be useful for quantifying the response of the embryo.

Key Words: chicken, genetics, breeder flock, prenatal stress, corticosterone

59 The effect of combined monochromatic lighting on sperm quality of broiler breeder males. Y. Bartman*[1], N. A. Cohen[1], L. Dishon[1], S. Zaguri[1], T. Porter[2], and I. Rozenboim[1], [1]*Hebrew University of Jerusalem, Rehovot, Israel, Israel,* [2]*University of Maryland, College Park, MD.*

Artificial targeted illumination has a pivotal role in reproductive processes in poultry. Light absorption mechanism in poultry consists of 2 main components: the eye (retinal photoreceptors) and the extraretinal photoreceptors located in the brain. Previous studies showed that photostimulation of brain extra-retinal photoreceptors elevate reproductive activities, while retinal photostimulation suppress reproduction. All those studies were conducted on female chicken. In this study, we tested the effect of combined monochromatic artificial photostimulation on reproductive activities of broiler breeder males. Fifty broiler breeder roosters (Ross), at 21 wks of age, were divided to 5 light treatment environmental and light control rooms (n = 10) equipped with individual cages. Light treatments: White (Control), long day (14 h) of blue light (480 nm) combine with a short day (6 h) of red light (630nm) (blue-red), long day of red light combined blue light (red-blue), long day of green light (560nm) combined with short day of red light (green-red) and long day of red light and a short day of green light (red-green). LED lamps at intensity of 0.1 W /m2 at bird head level provided all lighting. Each week until 65 wks. of age semen samples were collected and analyzed for: volume (ml), motility (sample were ranked from 1 to 8 where 1 shows total lack of motility and 8 indicates excellent movement with turbulence), sperm count by a hemocytometer, and vitality of a sperm by Eosin-Nigrosine staining. Furthermore, once a month blood samples were drawn for plasma hormonal assay. At 65 wks of age roosters were killed and tissue samples (hypothalamus, pituitary gland, retina and testes) were taken for mRNA gene expression of GnRH-I, LH and FSH, and steroid respectively. Semen motility in roosters from red-green group was significantly elevated over all the other light treatment groups ($P < 0.05$) during most of the experiment. Semen volume in red-green and blue-red groups were higher than all other groups. Semen concentration per ml was similar in all groups however when tested as concentration per ejaculation we could see a trend for higher concentration in the red-green group ($P > 0.05$). Testes weight was higher in the red-green treatment group than in all other treatments ($P > 0.05$). There were no significant differences in viability and we were able to see that the various light treatments didn't cause any effect on viability. Further endocrine and genomic assay are conducting to understand the mechanism of this phenomena.

Key Words: photostimulation, sperm, broiler

60 Energy intake by broiler breeder pullets stimulated reproductive hormones and fatness at the onset of lay. S. H. Hadinia*[1], G. Y. Bédécarrats[2], and M. Zuidhof[1], [1]*University of Alberta, Edmonton, AB, Canada,* [2]*University of Guelph, Guelph, ON, Canada.*

The effect of ME intake (MEI) on reproductive hormones and carcass characteristics at the onset of lay was evaluated in broiler breeder pullets. A total of 140 Ross 308 broiler breeder pullets were randomly assigned to 2 treatments in 4 pens of 35 birds each, and fed using a precision feeding (PF) system from 22 to 26 wk of age: 1) Low energy diet fed restricted using a typical commercial diet (2,807 kcal/kg, Low MEI) 2) High energy diet fed ad libitum (3,109 kcal/kg, High MEI). The PF system individually fed High ME treatment birds every time they came for feed, and Low MEI treatment birds only when their BW was less than the target BW. Hours of daylight was increased from 8 to 14 h at 22 wk of age with a light intensity of 30 lx. Palpation was used to detect sexual maturation via the presence of a hard-shelled egg in the shell gland. At the time of first egg, expression of gonadotropin releasing hormone (GnRH-I) and gonadotropin inhibitory hormone (GnIH) genes in the hypothalamus, and GnRH-I receptors (GnRH-RI, GnRH-RIII) and GnIH receptor (GnIH-R) genes in the anterior pituitary gland of each pullet was evaluated using RT-PCR. Blood samples were taken weekly and luteinizing hormone (LH), follicle stimulating-hormone (FSH) and 17-β-estradiol (E_2) determined using commercial ELISA kits. Each individual whole carcass was pressure cooked and homogenized for determination of CP and fat content. Data were analyzed using the GENMOD procedure in SAS. Pullets in High MEI treatment had higher MEI compared with pullets in Low MEI treatment (493 and 304 kcal/d respectively, $P < 0.001$). Moreover, pullets in High MEI treatment had 3-fold higher GnRH-I mRNA levels in the hypothalamus and approximately 1.5-fold higher GnRH-RI mRNA and pituitary GnIH-R mRNA expression was 0.67 of the Low MEI treatment ($P < 0.05$). However, MEI did not affect GnIH and GnRH-RIII expressions ($P > 0.05$). For High MEI and Low MEI treatments, respectively, from 22 to 26 wk of age, LH concentration was 3.16 and 1.65 ng/mL ($P < 0.004$); FSH concentration was 147.37 and 90.05 pg/mL ($P = 0.005$); E_2 concentration was 421.54 and 275.64 pg/mL ($P = 0.03$); carcass lipid was 13.81 and 10.46% ($P < 0.001$), and carcass protein was 19.89 and 20.65% ($P = 0.03$). The onset of lay for pullets in High MEI treatment advanced 100% were laying by 26 wk of age, compared with 50% in the Low MEI treatment ($P = 0.009$). In conclusion, it appears that higher MEI activated the hypothalamic-pituitary axis and increased body fat deposition, consequently stimulating reproductive hormone levels which advanced sexual maturation in broiler breeder pullets.

Key Words: reproduction, gene expression, hormonal trends, carcass compositions, broiler breeder pullets

61 Estradiol-17β levels demonstrate advanced sexual maturation in higher body weight broiler breeders on shorter rearing photoperiods. S. van der Klein*[1], S. H. Hadinia[1], G. Y. Bédécarrats[2], and M. Zuidhof[1], [1]*University of Alberta, Edmonton, AB, Canada,* [2]*University of Guelph, Guelph, ON, Canada.*

Body weight (BW) and rearing photoperiod are known signals affecting sexual maturation in broiler breeders, however, their combined effect on plasma estradiol-17β (E2) levels is unclear. The aim of this study was to investigate the effects of BW and rearing photoperiod on the timing of prepubertal increase in E2 levels after photostimulation (PS). Groups of 26 Ross 708 broiler breeder pullets were reared in 6 chambers using precision feeding stations, which controlled feed intake to achieve the target BW of each individual hen. Half of the pullets in each chamber were assigned to the breeder-recommended target BW curve (Std), and half to an accelerated target BW curve reaching the 21 wk BW at 18 wk (High). Pairs of chambers were randomly assigned to 8L:16D, 10L:14D, or 12L:12D light schedules and photostimulated at 21 wk, achieved by a single step to 16L:8D. To determine age at first egg (AFE), hens were palpated daily from wk 20 to 54 to identify the presence of hard-shelled eggs in the shell gland. Hens that did not commence egg production for the duration of the experiment were excluded from the analysis. From wk 20 to 28, blood samples were taken weekly from the brachial vein of 6 randomly selected birds per interaction. After ethanol extraction, E2 levels were measured with a commercial ELISA kit. The NLMIXED procedure in SAS was used to fit a sigmoidal curve to the pubertal increase in plasma E2, where $E2 = E_0 + E_{max}(\exp^{-\exp(-b(age-tinf))}) + \varepsilon$ and $tinf = \ln(-\ln((E_{max}-E_0)/E_{max}))/b + (t_0+u)$. E_0 was the prepubertal E2 baseline, E_{max} was the E2 level after sexual maturation, t_0 was the average age at the start of the prepubertal E2 increase from the baseline E_0, and a bird-specific random variable $u \sim N(0,V_u)$ associated with t_0

was included. None of hens in the 12L × Std treatment commenced egg production in the period E2 was measured, therefore, a one-way ANOVA was performed on *u* to determine treatment differences. E_0 was 331 pg/mL, E_{max} was 736 pg/mL, and average t_0 was 157.1 d. In the 8L × Std treatment t_0 occurred later (+1.29 d) compared with the 10L × High treatment (−2.00 d; $P = 0.003$). The 8L × High and 10L × High treatment tended to have a shorter time between t_0 and AFE compared with the 12L × High treatment (12.0 and 11.7 d, respectively vs. 26.3 d; $P = 0.066$). Time between PS and AFE was shorter in the High BW treatment compared with the Std BW treatment, and depended on rearing photoperiod (42.4, 24.8 and 69.9 d vs. 74.5, 100.8, and 158.8 d, for the 8L, 10L and 12L light schedules, respectively; $P = 0.034$). Increasing BW and shortening rearing photoperiod in broiler breeders advanced the prepubertal increase in E2 levels after PS.

Key Words: modified Gompertz curve, photorefractoriness, onset of lay, lighting, reproduction

62 Differential protein expression correlated with *Gallus gallus* sperm mobility. Z. Jarrell*, M. Ahammad, and A. Benson, *University of Georgia, Athens, GA*.

Genetic selection focused on rapid growth and high feed efficiency has resulted in broilers able to reach market weight in just 5 weeks. This pursuit in selection, while extremely successful, has had deleterious effects on the fertility of broiler breeders. The necessity for higher yielding birds will maintain, therefore there is a need for breeder management practices directed toward regaining fertility in the parent stock. At present, one of the primary assessments of male breeder fertility is quantification of sperm mobility, however little is known of the proteins which contribute to this function. Marker assisted selection is a very practical method for solving this fertility issue, but its development and implementation relies on identification of dependable biomarkers to inform selection. The objective of this study is to identify and characterize proteins involved in avian sperm mobility to elucidate potential biomarkers of male broiler breeder fertility. This study relies on analysis of differential expression of proteins between sperm samples separated by degree of mobility. Sperm samples collected via massage method from a population of 25 Athens-Canadian Random Bred roosters were separated via Percoll density gradient (PDG) centrifugation. The established PDG consisted of 1.08 and 1.07 g/cm^3 Percoll solutions, thereby establishing low, medium and high mobility groups post-centrifugation. Samples were then lysed via sonication in an isoelectric focusing (IEF) compatible urea/thiourea lysis buffer and concentrations standardized using Lowry protein assay. For each mobility group, 5 Bio-Rad 3–10pH immobilized pH gradient strips were rehydrated in urea/thiourea rehydration buffer containing 340µg in 125 µL of sample, subjected to IEF using the Bio-Rad PROTEAN i12 IEF system and equilibrated, all according to standard, Bio-Rad supplied protocol for 2-dimensional electrophoresis (2-DE). Strips were added to Bio-Rad Stain-Free gels and the 2nd dimension separated via SDS-PAGE. Gels were imaged via tryptophan UV-Vis spectroscopy (stain-free technology) using the Bio-Rad ChemiDoc^{TM} MP imaging system. Proteins expressed differentially between mobility groups with 95% confidence were initially identified through analysis by Bio-Rad PDQuest software. One-way ANOVA of the 23 differentially-expressed proteins identified by PDQuest revealed 8 individual proteins with significant decreases in expression as mobility increased (p-value = 0.02453). We therefore conclude these 8 proteins to be significant candidates for further characterization as biomarkers of decreased mobility in avian sperm.

Key Words: fertility, mobility, 2-D electrophoresis, broiler breeder, reproductive physiology

63 Altered expression of lactate dehydrogenase and monocarboxylate transporter involved in fermentative glycolysis in broiler wooden breast. D. Zhao*[1], M. Kogut[2], K. Genovese[2], M. Farnell[1], J. Lee[1], and Y. Farnell[1], *[1]Texas A&M University, College Station, TX, [2]USDA-ARS, College Station, TX.*

Wooden breast results in significant losses to the broiler industry due to reduction in meat quality. Intensive broiler growth rates are considered to be a major factor in the disease, which is indicative of abberant metabolic pathways. Lactate is the end product of glycolysis and is used as an energy source for muscles. Lactate dehydrogenase (LDH) is composed of tetramers derived from 2 LDH protein subunits, encoded by the LDH-A which converts pyruvate to lactate and LDH-B genes which converts lactate to pyruvate. Movement of lactate is facilitated by 2 major monocarboxylate transporters (MCTs), MCT-1 and MCT-4. Micro RNA (miRNA) is small fragment no-coding RNA that is a negative regulator in protein production. MiRNA-375 has been reported to be inversely associated with LDH-B expression. Therefore, the objectives of this study were to evaluate whether there were changes of LDHs, MCTs and miRNA-375 in wooden breasts compared with normal broiler breasts. Wooden (n = 8) and normal breasts (n = 8) were assessed by severe degrees of white stripping and hardness. Samples were collected from the upper left quadrant of the pectoralis major and stored in RNA-later[å]. Real-time reverse transcription PCR (RT-PCR) and Western blots were performed to investigate the level change of LDHs and MCTs in both mRNA expression and protein levels. Statistical analysis was determined via a student's *t*-test and significant differences were determined at $P < 0.05$. The mRNA level of LDH-A was 1.72-fold lower ($P < 0.0001$) in wooden breast tissues than those from normal breast tissue. In contrast, the mRNA level of LDH-B was 3.21-fold higher ($P < 0.0002$) in wooden breast than in normal breast. Wooden breast exhibited 13-fold higher ($P < 0.003$) LDH-B protein levels compared with normal breast muscle. However, the LDH-A protein levels in wooden breast muscle showed no significant change. The level of miRNA 375, which has been demonstrated to be inversely associated with LDH-B protein levels in human cancer cells, was found to have lower levels ($P < 0.025$) in wooden breast muscle compared with normal breast levels. The mRNA levels of MCT-1 and 4 were 2.30-fold and 2.84-higher ($P < 0.02$) than those in normal breast muscle, respectively. The expression levels of MCT-4 protein were elevated (3.5-fold, $P < 0.005$) in wooden breast. However, this upregulation was not observed with MCT-1 protein expression level in wooden breast. These data suggest that 2 metabolic pathways involved in fermentative glycolysis have been altered in broilers exhibiting wooden breast.

Key Words: wooden breast, lactate dehydrogenase, monocarboxylate transporters, miR375, fermentative glycolysis

64 Quantum Blue supplementation reduces the severity of woody breast myopathy in broiler chickens. J. Flees*[1], C. Coy[2], E. Greene[1], N. Anthony[1], S. Rochell[1], M. Kidd[1], C. Walk[3], S. Velleman[2], and S. Dridi[1], *[1]University of Arkansas, Fayetteville, AR, [2]The Ohio State University, Wooster, OH, [3]AB Vista, Marlborough, United Kingdom.*

The incidence of woody breast (WB) is increasing on a global scale representing a significant welfare problem and economic burden to the poultry industry and for which there is no effective treatment due to its unknown etiology. This study aimed to determine the effect of Quantum Blue (QB) super-dosing on growth performance and WB incidence in broilers and to define its underlying mechanisms. Male broiler chicks (Cobb500, n = 576) were randomly distributed into 48 floor pens and subjected to 6 treatments (12 birds/pen; 8 pens/treatment): a nutrient adequate control group (PC), the PC supplemented with 0.3% myo-inositol, a negative control (NC) deficient in available P and Ca by 0.15 and 0.16%, respectively, and the NC supplemented with QB at 0.01, 0.02, and 0.04% of feed. Feed intake (FI) was recorded daily, and individual body weights (BW) were recorded weekly. Birds were processed on d57, carcass traits were measured, and WB was scored based on a 0.5 grade scale and was categorized as NORM (0), MOD (0.5–2), and SEV (2.5–3). Breast samples from each treatment were taken from NORM (cranial region) and SEV (cranial region (Woody-Woody/WW) and caudal region (Woody-Normal/WN)) and snap frozen in liquid nitrogen. Gene expression were determined by reverse transcription-qPCR. Data were analyzed using one-way ANOVA and means were separated by Tukey's multiple comparison test and $P < 0.05$ was considered significant. The high dose of QB improved FCR, breast weight, and reduced fat pad size compared with the PC group. Although the overall incidence of WB did not differ between groups, high dose of QB reduces SEV WB by 5% compared with PC group. Metabolome analysis showed that QB treatment increased plasma free fatty acid (NEFA) levels which may explain the reduction in fat weight. Molecular analysis shows that hypoxia-inducible factor (HIF-1α) expression increased in WB compared with healthy birds and in avian myoblast (QM7) cells exposed to hypoxia compared with normoxia, suggesting that WB might due to a hypoxic status. Exposing primary satellite cells (SC) to hypoxia downregulated the expression of paired box (PAX7, PAX3), myogenic regulatory factor (MRF4), and myogenic factor (Myf5), SC and myogenic specific transcription factors, and upregulated the expression of fatty acid synthase (FAS), rate limiting gene in lipid synthesis, which indicates an altered fate of SC and loss of regeneration/repair of muscle fibers.

Key Words: Quantum Blue, woody breast, growth performance, gene expression, satellite cells

65 Neuropeptide Y characterization and regulation in chicken muscles. S. Hamad*[2], M. Zampiga[1], E. Greene[2], C. Shawhan[2], F. Sirri[1], and S. Dridi[2], *[1]University of Bologna, Bologna, Italy, [2]University of Arkansas, Fayetteville, AR.*

Neuropeptide Y (NPY) is a conserved neurotransmitter which consists of 36 amino acids and mainly expressed in the hypothalamus. NPY plays a key role in stimulating appetite and inducing feed intake. Recent research showed that NPY and its receptors (NPYRs) can be expressed by peripheral tissues, but their role is not well defined yet. As peripheral NPY system is still unknown in chickens, the objective of this study is therefore to characterize NPY and NPYRs and determine its hormonal and nutritional regulation in chicken muscle. Samples of breast muscle, leg muscle and hypothalamus were collected from 9-d-old male broiler chickens reared under standard conditions. Quail leg muscle and Quail myoblast cells were also used for characterization. For regulation, 16 broilers were divided to 2 body-weight matched group and subjected to 2 nutritional states: fed vs fasted for 24h. In a separate trial, body-weight matched broilers were IP-treated with different doses of chicken recombinant NPY (0, 60, 120 and 240 μg/kg BW). Data were analyzed

by student's *t*-test or One-way ANOVA with SNK multiple comparisons test. Significance was considered at $P < 0.05$. PCR and Western blot analyses showed that NPY and its related receptors were expressed in hypothalamus, breast muscle and leg muscle. Fasting significantly upregulated the expression of muscle NPY and NPYRs compared with the fed group. NPY administration induced the expression of NPY, NPYR5 and NPYR6 in chicken muscle compared with the untreated group. Together, our data showed that NPY system is expressed in avian muscle and it is regulated by the endocrine system as well as by the nutritional status.

Key Words: neuropeptide Y (NPY), neuropeptide Y receptor, breast muscle, leg muscle, chicken

67 Proteome and microbiota analysis reveals alterations of liver-gut axis under different stocking density of Peking ducks. Y. Wu*[1], J. Li[2], X. Qin[2], Z. Xiao[1], X. Dong[1], and J. Yuan[1], *[1]China Agricultural University, Beijing, China, [2]Shanxi Agricultural University, Jinzhong, China.*

The aim of this study was to determine the impact of stocking density on liver proteome and microbiota of Peking ducks. A total of two thousand 24-d old ducks were randomly allotted into 5 stocking density groups of 5 ducks/m^2, 6 ducks/m^2, 7 ducks/m^2, 8 ducks/m^2, 9 ducks/m^2, with 6 replicates for each group. On the 42 d of age, duck serum and pectorals were collected to determine biochemical indices such as total antioxidant capacity (T-AOC), malondialdehyde (MDA) and low-density lipoprotein cholesterol (LDL-C) by using commercial analytical kits according to the manufacturer's recommendations (Jian Cheng Bioengineering Institute, Nanjing, China). The liver and cecal contents of ducks were collected for proteome and microbiota analysis by using the tandem mass tag (TMT)-labeled quantitative proteomics and 16S rDNA sequencing, respectively. Curve estimation in SPSS (v.20.0, SPSS Institute, Chicago, IL) was used to assess the linear and quadratic effects of increasing stocking density. For TMT analysis, 2-sided *t*-tests were adopted to compare expression of proteins. A significance level of 0.05 was used for statistical testing. For 16S rDNA, linear discriminant analysis effect size (LEfSe) was applied to identify differentially abundant taxa between high and low stocking density groups. Serum MDA elevated while pectorals T-AOC decreased linearly with increasing stocking density. Proteome analysis revealed that high stocking density increased chronic liver disease related protein like metallothionein while diminished anti-oxidant related proteins such as regucalcin and catalase. High stocking elevated lipid metabolism-related proteins including Acyl-CoA dehydrogenase long chain (ACADL), Acyl-CoA synthetase family member 2 (ACSF2) and Acyl-coenzyme A oxidase (ACOX1). Besides, serum LDL-C enhanced linearly with density incrementing. In 16S rDNA analysis, a lower diversity was observed in high raising group. High stocking density also caused a depletion of anti-inflammation related bacteria metabolism related bacteria including *Bacteroidales, Butyricimonas*, and *Alistipe*. In addition, and decreasing bile acid metabolism-related bacteria such as *Ruminococcaceae, Clostridiales* and *Desulfovibrionaceae* in the high-density group. Both proteome and 16S rDNA results showed inflammation and chronic liver disease trend in high density group, which suggests the involvement of the liver-gut axis in oxidative stress. High stocking density would cause dysregulation redox and lipid metabolism in terms of serum, proteome and microbiota.

Key Words: duck, stocking density, stress, proteome, microbiota

68 Molecular detection of *Heterakis gallinarum* in earthworms and darkling beetles. K. Cupo* and R. Beckstead, *North Carolina State University, Raleigh, NC.*

Blackhead disease, caused by the protozoan parasite *Histomonas meleagridis*, is common in poultry production units causing up to 30% mortality in chickens and up to 100% mortality in turkeys. Previous research has focused directly on *H. meleagridis* transmission. This parasite is an anaerobic microorganism and cannot survive outside of a host for an extended period of time. *Heterakis gallinarum* has been shown to be an indispensable vector for *H. meleagridis*. Eggs of *H. gallinarum*, which most often are infected with *H. meleagridis,* can survive in the environment for up to 3 years. There are aspects of the epidemiology of blackhead disease that remain unknown, such as intermediate hosts carrying *H. gallinarum*. Identifying these intermediate hosts provides targets for biosecurity intervention. Earthworms are a known intermediate host for *H. gallinarum*. To determine the percentage of earthworms carrying *H. gallinarum* in a backyard chicken flock, DNA was isolated from small tissue samples from 7 earthworms and subjected to diagnostic PCR using primers specific for *H. gallinarum* sequence. Five of the samples were positive for *H. gallinarum*. This primer set, which has previously been shown to be specific for *H. gallinarum*, is able to detect the nematode in intermediate hosts. It has been suggested but not shown that darkling beetles may transport *H. gallinarum*. We hypothesize that darkling beetles act as intermediate hosts for *H. gallinarum* carrying *H. meleagridis*. To test this, DNA was isolated from 27 samples consisting of 5 darkling beetles collected from broiler breeder houses that have a history of blackhead disease. PCR analysis was run on each sample using the same primers as above. Twelve of the 27 samples were positive for *H. gallinarum*. The PCR diagnostic test was not set up to determine whether the parasite was attached to the surface of the darkling beetle or carried internally, nor can the test determine if the nematode is viable. These results suggest future infection trials are needed to confirm if the beetles are carrying viable *H. gallinarum*. A diagnostic PCR using primers for *H. meleagridis* was run on the DNA isolated from the darkling beetle samples. The PCR assay did not detect *H. meleagridis* in any of the samples. It is possible the darkling beetles are positive for *H. gallinarum* and not *H. melearidis*. It is more likely that the level of *H. meleagridis* DNA in the samples is below the sensitivity level of the primer set. Future assays need to be designed to increase the sensitivity of the assay or increase the DNA yield for *H. meleagridis*. Additionally, the potential of other insects found around poultry facilities to carry *H. gallinarum* will be evaluated using the diagnostic PCR.

Key Words: *Histomonas meleagrids*, *Heterakis gallinarum*, darkling beetle, earthworm, PCR

Student Competition: Management and Production II

69 Precision feeding: Watching individuals in the young flock, one drop at a time. J. A. Chew*, C. Ouellette, and M. Zuidhof, *University of Alberta, Edmonton, AB, Canada.*

Flock uniformity is a major objective when managing broiler breeders; lighting and feeding management decisions affect more birds similarly when birds are similar in body weight (BW). The poultry precision feeding (PF) station designed at the University of Alberta feeds birds on an individual basis, matching nutrient supply to nutrient requirements of discrete birds. The PF station has achieved 100% flock uniformity with a 1% coefficient of variation. However, some broiler breeders are slower than others to learn to eat from the stations. Once they understand the PF system, delayed learners compensate by eating more until their BW reaches the target BW, however slow learning can decrease BW uniformity during the early stages of growth. Identifying such birds would be a challenge for producers who manage thousands of birds at a time. The objectives of the study were to 1) incorporate a marker system with a PF station to mark birds with a non-toxic dye while they eat, allowing unmarked birds to be identified for intervention, and 2) to correlate the intensity of the markings with feed intake (FI), average daily gain (ADG), and number of visits as indicators of feeding success. A total of 40 broiler chicks were fed with a PF station equipped with a marker system from 0 to 21 d of age. Birds were trained to use the station from 0 to 6 d by following trails of feed leading up to the station. At 7 d, individual birds were identified with a radio frequency identification (RFID) wing band. The marker system administered a drop of diluted livestock marking dye each time a bird ate from the feeder of the station. Dilution provided a means of increasing variation in color intensity over repeated marking events. Post study, birds were divided into 3 categories (Early, Mid, and Late adopters) based on the rate at which they adapted to individual feeding from the PF station. Late adopters had the highest cumulative FI ($F > 0.07$) and lowest color intensity, cumulative dye, and meals from the PF station ($P \leq 0.05$); Early adopters had the lowest cumulative FI ($F < 0.07$) and highest color intensity, cumulative dye, and meals ($P \leq 0.05$); Mid adopters were intermediate. Stepwise regression confirmed our hypothesized relationship between color intensity, quantity of dye applied, cumulative FI, and cumulative number of visits to the PF station ($P \leq 0.05$). If the marking system can be scaled up successfully, it has potential as a commercial means of identifying of birds that require intervention, thus synchronizing the process of learning to eat from the PF system and ensuring a uniform start.

Key Words: precision livestock feeding, agricultural engineering, behavior, uniformity, feed intake

70 Growth performance of broiler chicks provided an incubation photoperiod in combination with a production photoperiod. K. Graham*[1], X. Li[2], J. MacIsaac[1], and B. Rathgeber[1], *[1]Faculty of Agriculture, Dalhousie Unversity, Truro, NS, Canada, [2]Dalhousie University, Truro, NS, Canada.*

Manipulation of lighting during incubation can have an impact on the developing embryo and the bird throughout its life cycle. The objective of this study was to evaluate different incubation photoperiods with the combination of 2 production photoperiods on broiler performance. It was hypothesized that chicks provided a photoperiod during incubation would have an advantage in growth performance when placed in the production facility with a shorter day length. 5,888 broiler hatching eggs were incubated under 8 lighting treatments over 2 trials. Eggs were randomly placed on 8 trays (50 eggs/tray for trial 1 and 42 eggs/tray for trial 2) in each of the 8 incubators utilized for this study. The incubators assigned to light treatments were equipped with 4 white LED (4100k) light strips set at a light intensity of 250 lx. Two lighting treatments were used for the setting phase of incubation (0–17 d); a control of 0 h light and 24 h dark (0L:24D) and a light treatment of 12L:12D. On d 17, the eggs were transferred and exposed to 1 of 4 hatching phase lighting treatments for the remaining 3 d of incubation. The 4 treatments consisted of a control of 0L:24D, 12L:12D, 18L:6D and 24L:0D each in 2 incubators. Chicks were removed from the incubators on d 21 (512 h) and transferred to the production facility where they were placed by treatment in 24 pens/room (24 birds/pen in trial 1 and 21 birds/pen in trial 2). Two production lighting programs were used; a long day treatment of 23L:1D and a short day treatment of 18L:6D each in 2 rooms. Birds were fed a standard starter, grower and finisher diet throughout production. Chicks were weighed upon placement in pens and the body weights and feed consumption were recorded daily for the first 7 d and d 14, 25 and 33. Data were analyzed as a split plot design using the Proc Mixed Procedure of ANOVA. The incubation and production photoperiods did not impact body weight gain (476.1g) or feed consumption (480.4g) for the first 14 d. Body weight gain was significantly higher ($P < 0.05$) from 15 to 25 d for birds raised under 18L:6D at 1079.8g compared with 1001.2g for 23L:1D. Feed consumption was similar ($P < 0.05$) with 18L:6D at 1404.1g and 23L:1D at 1363.0g. Body weight gain from 26 to 33 d was significantly higher ($P < 0.05$) for birds raised under 23L:1D at 899.2g compared with 18L:6D at 818.8g. Again, feed consumption was higher ($P < 0.05$) at 1530.8g for 23L:1D compared with 1489.5g for 18L:6D. The results indicate that regardless of incubation photoperiod, broiler chickens had a higher body weight gain and feed consumption from 15 to 25 d with the 18L:6D treatment but required the longer day length from 26 to 33 d to meet their nutritional requirements.

Key Words: broiler, lighting, incubation, feed consumption, weight gain

71 Effects of colored LED light illumination during incubation on organ development and immune response of broiler chickens. X. Li*, B. Rathgeber, K. Graham, J. Henry, and J. MacIsaac, *Dalhousie University, Truro, NS, Canada.*

Providing photoperiod to the incubation environment has been shown to improve post-hatch development and reduce stress during the grow-out phase. However, the impact of providing light during incubation on broiler immune responses is still not known. An experiment was conducted to investigate the effects of providing different colors of photoperiodic lighting during incubation on immune organ development, growth performance and serum IgG concentration in broiler chickens following vaccination. A total of 1600 Ross 308 broiler hatching eggs were randomly distributed into 8 incubators with 4 lighting treatments. The incubation lighting treatments included: incubated under dark as control, illuminated with white, red or blue lights for 12 h (h) per day. After hatch, 16 males and 16 females from each treatment were euthanized to evaluate the organ development. 1056 broilers were then placed into 48 pens by the light color treatments and raised under an 18 h of light per day lighting regimen. On d 10 and 21 posthatch, 5 birds per pen were immunized intraocularly with AviPro ND-IB Polybanco vaccine. On d 10, 21, 25 and 35 posthatch, the development of organs (spleen, bursa of Fabricius and liver) were evaluated by the relative weight to chick weight and serum samples were analyzed by using commercial

chicken IgG ELISA kit for serum IgG response to the vaccine. The relative organ weight was calculated based on yolk free body weight (YFBW). Data were analyzed using the Proc Mixed procedure of SAS. For day-old chicks, YFBW, the relative weight of the spleen (RSW), bursa of Fabricius (RBW) and liver (RLW) were not affected by incubation lighting treatments ($P > 0.05$). On d 25 posthatch, the birds hatched from blue light had higher RLW than those from white light treatment. In addition, the chicks hatched under white light had higher serum IgG concentration than those hatched under red light ($P < 0.01$). However, the serum IgG levels were not affected by lighting treatments on d 10, 21 or 35. These results indicate that the chicken embryos illuminated with white LED light with photoperiod leading may be more sensitive and quicker response to a booster vaccine.

Key Words: Broiler, Incubation, Light, Organ development, Immune response

72 Effectiveness of turkey hatching egg sanitization with the combination of hydrogen peroxide and ultraviolet light. K. Cantu*, G. Archer, and C. Coufal, *Texas A&M University, College Station, TX.*

Turkey hatching eggs are routinely sanitized in commercial breeder operations to remove adhering organic material and reduce microbial loads that could negatively impact hatchability. The process often involves the use of a commercial egg washing machine followed by the application of a sanitizing agent such as quaternary ammonium. Previous studies with chicken and duck eggs have determined that hatching egg sanitization by the combination of hydrogen peroxide and UV light (H_2O_2/UV) resulted in lower eggshell microbial loads and improved hatchability. The objective of this study was to compare the effectiveness of the H_2O_2/UV method of egg sanitization to a commercial hatching egg washing method and evaluate eggshell microbial loads, embryonic mortality, and hatchability. One trial was performed to compare 3 treatments using commercial turkey hatching eggs. Treatments consisted of 1) control (untreated) eggs, 2) washed eggs that were treated by the supplier of the eggs, and 3) eggs sanitized with a prototype egg sanitizer which applies the H_2O_2/UV treatment in a commercially feasible manner. For each treatment, 10 eggs were aseptically sampled at random before incubation for enumeration of the total number of microorganisms on the eggshell surface using aerobic plate count (APC) Petrifilms. A total of 1,758 turkey eggs were placed into plastic flats and incubated in 9 incubators (3 incubators per treatment). Flats of eggs were weighed before placement and at time of transfer to hatchers to determine moisture loss. A one-way ANOVA was used to analyze all data ($P < 0.05$) with egg serving as the replicate for microbial data and incubator serving as the replicate for hatchability data. Results demonstrated that the average APC for control, washed, and sanitized treatments were 5.09, 3.03, and 1.76 $\log_{10}$cfu/egg, respectively, and the washed and sanitized eggs were significantly lower than the control eggs ($P < 0.05$). No differences were observed for embryonic mortality, egg moisture loss, or hatchability between treatments ($P > 0.05$). The data collected in this study demonstrated that using the combination of H_2O_2 and UV light as an egg sanitization method effectively reduced eggshell microbial loads with no detrimental impact to embryonic mortality or poult hatchability.

Key Words: turkey, hatching eggs, hatchability, hydrogen peroxide, UV light

73 Intermittent administration of probiotic lactic acid bacteria to broiler chickens during diet changes via drinking water improves feed efficiency and promotes populations of beneficial gastrointestinal bacteria. T. Broderick*[1], I. Hindrichsen[2], J. Lee[1], and T. Duong[1], *[1]Texas A&M University, College Station, TX, [2]Novozymes, Bagsvaerd, Denmark.*

Lactic acid bacteria (LAB) have been used widely as probiotics because of their beneficial health effects, and their administration has been demonstrated to improve animal health, growth performance, and microbial food safety in poultry production. However, their use has fallen out of favor because of their low survival during pelleting of feed. Shifts in feed ingredient profiles across diet phases alter fermentation substrates in the gastrointestinal tract and perturb the microbiota. Administration of probiotic LAB during diet changes may mitigate perturbations in the gastrointestinal microbiota and improve growth performance. Delivery through drinking water may ensure viability of LAB and facilitate intermittent administration when applied for this purpose. In this study, we evaluated the effect of intermittent administration of probiotic LAB through drinking water during diet changes on growth performance and gastrointestinal microbiota in broiler chickens over a 42 d production period. Broiler chickens were fed the same diet and administered either untreated water, 10^6 cfu bird^{-1}day^{-1} of LAB for one day post-diet change (Low), or 10^8 cfu bird^{-1}day^{-1} LAB for 2 d pre- and post-diet changes (High). Growth performance and bacterial count data were analyzed using ANOVA, and correlations between growth performance and bacterial counts were analyzed using Pearson's *r*. No significant differences in body weight, average daily gain, or feed intake were observed ($P > 0.05$). However, feed conversion ratio from d 0–27 post-hatch of broilers administered probiotic treated water was lower when compared with the untreated control ($P = 0.013$). More LAB were recovered from broilers administered the high probiotic dose at d 15 post-hatch ($P < 0.001$), while fewer *Campylobacter jejuni* were recovered at d 41 from broilers administered the low probiotic dose ($P = 0.001$). Additionally, intermittent LAB administration reversed the reduction in total LAB after diet changes across the starter to grower diet change ($P = 0.012$). Negative correlations were observed between day13–27 FCR and LAB counts on d 15 ($r = -0.332$, $P = 0.073$), 29 ($r = -0.431$ $P = 0.018$), and 41 post-hatch ($r = -0.443$, $P = 0.014$). Cumulative FCR was also negatively correlated with LAB counts on d 29 ($r = -0.409$, $P = 0.025$) and 41 post-hatch ($r = -0.312$, $P = 0.094$). *Clostridium perfringens* counts on d 41 were positively correlated with d 13–27 FCR ($r = 0.357$, $P = 0.053$) and cumulative FCR ($r = 0.433$, $P = 0.017$). Our results suggest that administration of probiotic LAB in drinking water improves feed efficiency and promotes positive shifts in the gastrointestinal microbiota during diet changes in broiler chickens.

Key Words: lactic acid bacteria, probiotics, water supplementation, feed conversion

74 The effect of refined functional carbohydrates from enzymatically hydrolyzed yeast on coccidiosis-vaccinated broilers fed diets containing coarse or fine corn. C. Caraway*[1], G. Walker[2], S. Jalukar[3], and J. Brake[1], *[1]North Carolina State University, Raleigh, NC, [2]College of Veterinary Medicine, North Carolina State University, Raleigh, NC, [3]Church and Dwight, Mason City, IA.*

The interaction between corn particle size and feed additives as it pertains to broiler live performance has received little attention. This study was conducted to evaluate the effects of corn particle size and refined functional carbohydrates (RFC; Aviator SCP, Arm and Hammer Animal Nutrition, Princeton, NJ) on live performance and *Salmonella* prevalence in coccidiosis vaccinated broilers. The following treatments were applied: 1) Fine Corn (FC), 2) coarse corn (CC), 3) FC+RFC, 4) CC+RFC, and 5) CC+SAL (salinomycin; 60 g/MT). For CC treatments, CC replaced 0%, 15%, 30%, and 45% of FC in the prestarter, starter,

grower, and finisher diets, respectively. The target d_{gw} (average particle size) of FC and CC was 300µm and 1400µm, respectively. RFC was supplied at 100 g/MT in appropriate diets. Treatments were assigned to 40 pens with 8 replicate pens per treatment. Feed consumption, BW, and FCR were determined at 10, 19, 35, and 48 d. Gastrointestinal tract measurements including weight, pH, and cecal *Salmonella* prevalence were determined at 19, 35, and 48 d. Live performance and organ measurements were analyzed using JMP 13.2 and *Salmonella* prevalence data were analyzed using PROC FREQ of SAS 9.4. Through 10 d, broilers fed RFC exhibited increased BW gain ($P \leq 0.05$) and improved FCR ($P \leq 0.01$) compared with broilers fed SAL. A natural necrotic enteritis outbreak occurred at 12 d of age and mortality was significantly impacted by dietary treatments. Increased NE mortality was observed in broilers fed FC+RFC (6.5%) and CC+RFC (9.6%) compared with broilers fed CC+SAL (0.5%; $P \leq 0.05$). At 19 d, decreased proventricular pH was observed in broilers fed RFC ($P \leq 0.05$) and there was a trend for reduced duodenal pH in broilers fed CC ($P \leq 0.10$). Greater than 50% of all broiler ceca were *Salmonella*-positive at 19 d, but the use of RFC was shown to decrease cecal *Salmonella* prevalence at 48 d ($P \leq 0.05$). Although differences in early mortality were observed, vaccinated broilers fed CC or CC+RFC exhibited similar BW and FCR at 48 d as broilers fed CC+SAL. In contrast, broilers fed FC or FC+RFC exhibited significantly poorer FCR compared with CC+SAL through 48 d ($P \leq 0.05$). These data suggested that CC use after 10 d may provide value in an antibiotic-free system by ameliorating live performance losses associated with coccidiosis vaccination. These data also demonstrated that RFC are capable of mitigating *Salmonella* colonization in coccidiosis-vaccinated broilers following a natural NE outbreak. Further research is warranted to investigate the interactions between CC, RFC, and coccidiosis.

Key Words: particle size, coccidiosis, necrotic enteritis, yeast, antibiotic-free

75 Increased body weight in broilers from higher body weight hens suggests an epigenetic mechanism. K. Humphreys*, S. van der Klein, M. H. Amaleh, F. Robinson, and M. Zuidhof, *University of Alberta, Edmonton, AB, Canada*

Epigenetics refers to heritable changes in gene expression that are not due to a DNA sequence change. Epigenetic changes arise when parental environmental conditions alter their gene expression in such a way that those alterations are passed on to offspring, allowing progeny to cope with similar environments. The objective of this study was to determine if maternal feed availability influenced offspring performance. Broiler breeders are intensively feed restricted and may pass that environmental information to their offspring. We therefore hypothesized that 1) relaxing maternal feed restriction would increase growth efficiency in broilers and, 2) increased feed efficiency would be seen in feed restricted broilers from feed restricted hens. This experiment used offspring from 2 maternal treatments, 2 sexes and 3 feeding treatments. Maternal treatments consisted of Ross 708 hens raised on standard breeder-recommended target BW (SBW), or 121% of SBW (HBW). Ross 708 broiler offspring (n = 264) were placed into 72 pullet cages from 0 to 42 d of age. These broilers were fed ad libitum until d 28. From 29 to 42 d of age, they were provided feed ad libitum (AL), or at 80 or 60% of AL. Individual BW and pen level feed intake were recorded weekly. At 28, 35 and 42 d of age, body composition was determined after dissection. Data were analyzed using a mixed model in SAS. HBW offspring had an ADG of 57.6 g/d, which was 2.8% greater than that of SBW offspring (56.0 g/d; $P = 0.034$). The 42 d BW of the HBW offspring (2,486 g) was 3.9% greater than SBW offspring (2,388 g; $P = 0.046$). HBW offspring had 6.6% heavier gut weights than offspring of standard BW hens ($P = 0.006$). Increasing feed intake of broiler breeder hens increased the growth performance of the offspring. In agreement with our first hypothesis, HBW offspring had a higher ADG and were heavier than SBW offspring at 42 d of age. Offspring from HBW hens had larger gut weights, which may have allowed them to absorb more nutrients, increasing their ADG and final BW. No maternal treatment difference was found between feeding treatment, therefore the 2nd hypothesis was rejected. Epigenetic mechanisms that alter the expression of gene associated with growth rate would provide a reasonable explanation for the differences observed.

Key Words: epigenetic, broiler, feed restriction, nutrigenomics, growth potential

76 Effect of corn particle size and proportion of fines on growth performance of broiler chickens reared to 21 days of age. J. Powell*, O. Tejeda, J. Starkey, and C. Starkey, *Auburn University, Auburn, AL.*

Factors such as ingredient particle size, formulation, conditioning, pellet die speed, and die specifications can impact feed quality. Previous research has demonstrated that broiler chickens require more time to reach proper feed intake when consuming diets in meal form as opposed to those in pelleted form. The feed manufacturing and transport process generates fines which are then delivered to the broilers during the rearing period. During the first 3 weeks of life, broiler growth rate is most rapid and feed intake must be sufficient for BW gain. If birds cannot consume enough feed, BW gain will decrease. The objective of this 2 × 3 experiment was to determine whether different corn particle size and proportions of fines in the feed affected broiler chicken growth performance. A common corn and soybean-meal based broiler starter feed was manufactured using corn ground to 1,680 µm (LARGE) and 841 µm (SMALL). All diets were formulated to meet or exceed the nutrient recommendations of the primary breeder. Corn of each particle size was mixed with whole pellets to achieve treatments with 25%, 50%, and 75% fines. Commercial, day-of-hatch, Yield Plus × Ross 708, female broiler chicks (n = 288) were randomly allotted to 1 of 6 feed treatments and housed in Petersime battery cages with 6 birds per cage. Birds and feed were weighed once a week to determine BW gain (BWG), feed intake (FI), and FCR. Data were analyzed using the MIXED procedure of SAS. Means were considered significantly different when $P \leq 0.05$. Over the 21-d rearing period, no significant differences in BWG ($P = 0.7123$), FI ($P = 0.3226$), or FCR ($P = 0.5379$) were observed among birds fed SMALL and LARGE particle size corn. From d 8 to 14, BW gains for birds consuming diets including 25% and 75% fines were greater when compared with birds consuming 50% fines ($P = 0.0026$). From d 0 to 21, FI was greater for birds fed 75% fines compared with those fed 25% and 50% fines ($P = 0.0341$), while BWG was greater for birds fed 25% and 75% fines when compared with those consuming 50% fines ($P = 0.0030$). Overall, birds consuming only 25% fines had lower FCR than those consuming diets including 50% or 75% fines ($P = 0.0018$). The results of this experiment show that increasing the percentage of fines decreased overall broiler production efficiency.

Key Words: particle size, fines, pellet quality, broiler, growth performance

77 Evaluation of multiple random coefficients in a nonlinear mixed Gompertz model estimating growth parameters in two heritage chicken lines. M. Afrouziyeh* and M. Zuidhof, *University of Alberta, Edmonton, AB, Canada.*

Robust ability to predict the growth pattern of birds is necessary to optimize poultry production systems. Growth models describe BW changes over time, allowing information from longitudinal measurements to be combined into a few parameters with biological interpretation. Nonlinear mixed models (NLMM) allow for the inclusion of random factors. These factors can account for a relatively large subset of the total variance explained by within-bird measurement correlation. The aim of this study was to evaluate different NLMM using birds from 2 heritage chicken lines; New Hampshire (NH) and Brown Leghorn (BL). A total of 32 birds (16 mixed sex birds from each strain) were raised to 17 wk of age. After 12 wk, half were continued on ad libitum feed intake (AL), and half were pair-fed 95% of the AL intake of a paired bird closest in BW using a precision feeding system. Growth data of the birds were fit to a mixed Gompertz NLMM with a variety of different bird-specific random coefficients. The model had the form: $BW = Wm*\exp(-\exp(-b*(t- t_{inf})))$; $t_{inf} = \ln(-\ln(W0/Wm)/b$; where Wm was the mature BW, b was the rate of maturing, W0 was initial BW, t was age (d), and t_{inf} was the inflection point (d). This fixed effects model was compared with NLMM using the Akaike information criteria (AIC) to evaluate relative model suitability. Random variables $Wm_u \sim N(0,V_{Wm})$ and $b_u \sim N(0,V_b)$ were tested separately and together. Proc NLMIXED in SAS was used for the NLMM, and ANOVA of the residuals of mature BW and rate of maturing was done using MIXED procedure, and LSmeans were compared using Tukey test. The model with both random variables had lowest AIC and was therefore chosen as the best model, which predicted overall Wm = 2.00 ± 0.106 kg and b = 0.0273 ± 0.00046. For BL, random coefficients were $Wm_u = -0.299$ and $b_u = -0.00034$; for NH: 0.468 and 0.00028, respectively; for females: −0.324 and 0.00028; for males: 0.493 and −0.00035; for AL fed birds: 0.246 and −0.00117; for birds fed 95% of AL: −0.077 and 0.00110, respectively. These values should be interpreted such that Wm for the BL strain was 0.299 kg less than the overall mean Wm (2.00 kg), or 1.701 kg; correspondingly, Wm for the NH strain was 2.468 kg, and so on for all effects tested. NLMM coefficients allow stochastic prediction of BW of individuals at any age, allowing for unique new decision support modeling applications.

Key Words: Gompertz, growth, model selection, multiple random coefficients, nonlinear mixed model

78 The efficacy of an engineered biocarbon in young broilers during aflatoxin exposure. Haci Bayir*, J. Liu, C. Ritz, and J. Fowler, *University of Georgia, Athens, GA.*

This study was conducted to evaluate the efficacy of a carbonaceous biomass (Bio-C) in mitigating the effects of a 500 ug/kg dose of aflatoxins (AF). A total of 192 Cobb-500 male broilers were obtained on the day of hatch and randomly allocated to one of 32 treatments pens (6 birds/pen). Birds were fed a broiler starter mash diet containing either 0 or 500 ug/kg AF, with or without 0.4% of Bio-C, resulting in 4 treatments arranged as a 2x2 full-factorial. Pen weights and feed consumed were recorded at 0, 7, 14, and 21 d of age, and mortality was recorded daily. On d 21, 3 birds from each pen were killed by cervical dislocation, and the liver, kidney, and spleen were removed and weighed for relative organ assessment. Data were analyzed as a 2x2 full-factorial for AF level and Bio-C using the GLM procedures of SPSS, with significance defined at $P \leq 0.05$. The results showed that the performance of birds receiving 500 ug/kg AF were not significantly different compared with the control group at any point during the 21 d trial. No main effects were seen on the performance parameters by the inclusion of Bio-C in the diet. There was also no AF/Bio-C interaction. The relative weights of the liver, kidney, and spleen were not significantly different whether birds were fed 500 ug/kg AF or 0.4% Bio-C. These data suggest that supplementation of Bio-C has no effect on broiler performance when fed in diets free of AF or containing up to 500 ug/kg.

Key Words: aflatoxin, biocarbon, binder, broiler

79 Effect of late-stage embryonic thermal manipulation on hatch, growth, and meat quality of yield type commercial broilers. S. Orlowski*, J. Hiltz, C. Maynard, and N. Anthony, *University of Arkansas, Fayetteville, AR.*

Current commercial selection practices focus solely on selection for late stage hypertrophic (increase in muscle fiber size) growth to promote increased body weight and yield. Little research has been done to evaluate the effect of selection for growth at an earlier stage that focuses on hyperplastic growth or the increase in muscle fiber number and accumulation of nuclei through satellite cell proliferation. Thermal manipulation (TM) during embryogenesis has been shown to promote growth, possibly due to an increase in hyperplasia. The purpose of this study was to attempt to impact muscle growth and resultant myopathies through late stage embryonic thermal manipulation. Fertile eggs from a yield strain broiler were incubated to d14 and then separated into a control (C) (constant temperature of 37.8°C) and 2 TM treatments (n = 160/trt). The TM treatments consisted of either a 3 h (3) or 6 h (6) period of increased temperature (39.5°C) on each of embryonic d 15, 16 and 17. The impact of thermal manipulation on hatch window was captured by recording hatched chicks every 4 h. Commercial broiler diets (starter 0–3, finisher 3–6 weeks) were fed post hatch throughout the study. Growth was recorded and all birds were processed at d 47. Each bird was processed and evaluated for breast yield and subjectively scored for woody breast and white striping myopathies. Data were analyzed using a 2-way full factorial ANOVA (line/sex) and means were separated using Tukey's HSD. Hatch window revealed that both the 3 and 6 h treatment groups hatched earlier than the untreated control. The greatest hatch of fertile was in the 3 h (96.39%) and the lowest in the control (89.22%). Percentage breast was highest in the 3 h treatment (25.8%) with no difference between the C and 6 h (25.0 and 25.1% respectively). The woody breast and white striping scores did not differ between treatment groups. Overall FCR did not differ between the treatment groups. For this study, short periods of TM (3 h) helped improve breast yield with no negative impact on feed conversion or the incidence of muscle myopathies.

Key Words: broiler, thermal manipulation, meat quality, hyperplasia, muscle growth

80 Effects of diets containing salt versus bicarbonate and level of physical activity on white striping and woody breast. C. Wu*, D. Hodgson, M. Livingston, and K. Livingston, *North Carolina State University, Raleigh, NC.*

Wooden breast (WB) is a muscle myopathy with palpably hard breast tissue. Research has indicated birds that present with WB have shown symptoms of hypoxia. Replacing NaCl with $NaHCO_3$has shown reduced incidence of hypoxia in broiler chickens. Moreover, it is common for employees to walk through a broiler house to maintain broiler activity, stimulate feed consumption, and increase live performance. However, walking through a house can cause wing flapping, which may contribute increased incidence of WB. Therefore, we evaluated the effect of dietary NaCl vs $NAHCO_3$subjected to activity or no activity on the severity of WB. Six hundred male Ross 708 chicks were allotted to 1 of 4 treatments with 15 birds/pen and 10 replications per treatment. This resulted in a 2x2 factorial design: NaCl vs $NaHCO_3$diet and active vs inactive birds. The birds were fed a diet containing NaCl or $NaHCO_3$. One half of the treatments were disturbed 3x/day by walking through the pens, while the remainders were not disrupted. Individual body weights were recorded weekly, starting at 14d. At 28 and 35 d, blood was collected and analyzed (iSTAT hand-held analyzer) from 2 birds per pen. At 29 and 36 d, these birds were harvested to analyze yield and score for WB and white stripping (WS). Data were analyzed using PROC GLM and PROC MIXED procedures of SAS . Throughout the study, active birds had greater body weights than inactive birds ($P \leq 0.05$). In addition, no significant differences in regards to WB with diet or activity at 29 or 36 d of age were observed. At 28 d birds fed salt diets exhibited reduced PCV% ($P \leq 0.01$) and greater breast yield % ($P \leq 0.05$). At 35 d birds consuming $NaHCO_3$exhibited increased levels of total CO_2, HCO_3, and base excess in the extracellular fluid ($P \leq 0.05$) with increased WS score, decreased dressing %, and breast yield % ($P \leq 0.10$). Active birds exhibited increased leg yield % at 36 d ($P \leq 0.05$) with trending increase in breast muscle weight (g) ($P \leq 0.10$). These data demonstrated Na source and level of activity had no effect on WB, while $NaHCO_3$birds had more WS. Furthermore, more active birds exhibited greater body weight, breast weight, and leg yield.

Key Words: physical activity, wooden breast, white striping, blood, sodium bicarbonate

81 Myofiber morphology and serum enzyme concentrations as potential biomarkers for wooden breast myopathy in high yielding broiler chickens. H. Cordova-Noboa*, E. Oviedo-Rondón, S. Alvarez, A. Sarsour, L. Gross, I. Martinez, D. Sapcota, D. Lopez, V. San Martin, and J. Barnes, [1]*North Carolina State University, Raleigh, NC.*

Wooden breast (WB) myopathy has been associated with muscle fiber degeneration and fibrosis. However, limited data has been published regarding the effects of this disorder on breast myofiber morphology in heavy broilers and its relationship with serum enzymes that could be used as potential biomarkers for this myopathy. Consequently, the objective of this study was to evaluate the effects of WB on breast meat quality, differential cell blood count and clinical biochemistry, and myofiber morphology in *Pectoralis major* samples affected with diverse degrees of WB severity. After processing *P. major* muscle samples were submitted for meat quality evaluation and WB scoring in a 4-point based scale (1 = none, 2 = mild, 3 = medium, and 4 = severe). A slice of muscle from each sample was obtained for further histological measurements. Blood samples were collected the day prior slaughter to evaluate white (WBC) and red blood cell count (RBC), clinical hematology, enzyme concentrations (creatine kinase CK, alanine aminotransferase ALT, aspartate aminotransferase AST, gamma-glutamyl transpeptidase GGT and lactate dehydrogenase LDH), and mineral content (P, Ca, Na, K, and Cl). Each individual *P. major* sample was considered as the experimental unit. Data were analyzed using ANOVA to evaluate the levels of WB severity, and mean separation was performed by Tukey's test. Meat quality evaluation indicated that samples affected with severe WB had higher ($P < 0.05$) ultimate pH (6.03 *vs*. 5.87), L* (56.79 *vs*. 53.00), and b* (9.17 *vs*. 5.37) values compared with samples without WB (score 1). Results obtained from differential cell count showed no differences ($P > 0.05$) among WB scores on WBC, RBC, mean corpuscular volume, and hemoglobin. However, the highest serum ALT, AST and CK ($P < 0.01$) were associated with severe WB (score 4) when compared with samples with WB score 1. Histological myofiber measurements suggested that fillets affected with severe WB presented ($P < 0.01$) the lowest diameter (36.44 μm) and area (1,301 μm^2) among WB scores. In addition, severe WB presented greater ($P < 0.01$) area and diameter CV% of fibers within each sample. Interestingly, moderate negative correlations ($P < 0.001$) were observed between WB severity with muscle fiber area and diameter ($r = -0.65, -0.68$) and positive correlations with AST, ALT, LDH, and CK ($r = 0.63$, 0.54, 0.46, and 0.53, respectively). Likewise, moderate positive correlations ($P < 0.05$) were detected between L* with AST, LDH, and CK ($r = 0.40$, 0.33, and 0.42, respectively). In conclusion, it was demonstrated that severe WB had detrimental effects on breast meat quality and myofiber degeneration with impact on metabolic enzymes that could be explored as WB blood biomarkers.

Key Words: wooden breast, blood parameter, histological measurement, muscle fiber, broiler

82 Correlation of incidence of woody breast to white stripping in broilers. M. Aguirre*, C. Alvarado, H. Leyva-Jimenez, and J. Lee, *Texas A&M University, College Station, TX.*

Woody breast (WB) and white stripping (WS) have been previously associated with older and heavier birds. However, there is limited information supporting the correlation between these 2 muscle conditions and growth parameters. The objective of this study was to investigate the relationship between WB and WS using different growth production factors. A combined database of 4,332 broilers from several different research experiments conducted during 2016–2017 at Texas A&M University was used in this study. Parameters such as strain, sex and processing age (34, 46, 47, 49, 56 d) were evaluated. The data were analyzed using the multivariate analysis function of JMP Pro 13.0 and Pearson correlation coefficients were obtained for the correlation between WB and WS. Results indicated that there was a positive association between WB and WS when data was analyzed by sex (female = 0.64, male = 0.55, $P < 0.001$), by strain (Cobb-500 = 0.51, Cobb-700 = 0.52, Ross-708 = 0.48 and Hubbard99xCobb-500 = 0.40, $P < 0.001$), and by age (34d = 0.40, 46d = 0.37, 47d = 0.51, 49d = 0.51, 56d = 0.52, $P < 0.001$). For example project I compared Cobb-500 at 47 d processing age and the correlation was higher for females (0.58, $P < 0.001$) than males (0.30, $P < 0.001$). Project II compared Cobb-700 at 47d of age and the correlation was higher for females (0.60, $P < 0.001$) than males (0.43, $P < 0.001$). Project III compared Hubbard-99xCobb-500 at 34 d of age and the correlation was higher for females (0.52, $P < 0.001$)

than males (0.29, $P < 0.001$). Regardless of the strain and age, females appear to have a higher relationship on the incidence of WB and WS than males. Project IV and V compared males Cobb-500 at 49 d (0.50, $P < 0.001$), Ross-708 at 46d (0.37, $P < 0.001$), 49d (0.51, $P < 0.001$), and 56d (0.52, $P < 0.001$) of age was 0.50 ($P < 0.001$). Irrespective to the strain there is an increase in association of WB and WS as the birds processing age increased. Therefore, results suggest that overall there is a positive association between WB and WS in older broilers. Potentially other unknown factors can play a major role in the relationship of these 2 myopathies and further correlation research should be conducted.

Key Words: woody breast, white stripping, muscle conditions, meat quality

83 Evaluating shear wave elasticity imaging and ultrasonography as advanced methods to study woody breast. A. Smith*[1], R. Moon[2], L. Garner[1], and A. Morey[1], *[1]Auburn University, Auburn University, AL, [2]College of Veterinary Medicine, Auburn University, Auburn University, AL.*

As improvements to genetics, nutrition, and management have allowed broilers to reach higher body weights in shorter growth periods, several conditions have emerged that decrease breast meat value. Existing methods of rapidly and accurately detecting these conditions are not sufficient, necessitating the investigation of new technologies. Woody Breast (WB) was selected as a model for these conditions due to its pervasiveness, negative impact on meat quality, and lack of organoleptic detection technique. Shear Wave Elasticity Imaging (SWEI), also known as Elastography, has recently emerged as an ultrasonic technique to analyze the relative stiffness of target tissues. Therefore, the objective of this study was to examine the validity of using SWEI or ultrasound (US) as possible methods to assess the quality of raw poultry breasts, particularly the hardness and structural tissue changes associated with WB. To evaluate these methods, 30 raw boneless skinless breast filets fillets were collected and categorized into one of 4 WB severity categories, including non-woody, mild, moderate, and severe. This was replicated twice. Filets were weighed and evaluated using manual palpation techniques for the presence and severity of WB using a 4-point scale, then stored overnight at 4°C. The following morning, filets were scored again for WB severity and scanned using US/SWEI with a Toshiba Aplio 500 Ultrasound. Filets were then weighed to determine drip loss, cooked to an internal temperature of 165°F in covered pans, cooled, reweighed to determine cook loss, and stored overnight at 4°C. Filets were allowed to warm to room temperature and texture analysis was performed using the PC-BMORS method. Images generated by SWEI and US were analyzed using ImageJ software, and statistical data were analyzed using SAS one-way ANOVA at a significance of $P < 0.05$. Consistent with previous findings, cook loss values were higher with increasing WB severity, with an average loss of 32% for mild filets and 46% for severe filets. Clear visual differences in tissue architecture between filets were observed using US and SWEI, though numerical data generated by SWEI were not statistically significant. In conclusion, further investigation of SWEI and US may be warranted to determine whether it can be used as a reliable method of WB investigation.

Key Words: woody breast, shear wave elasticity imaging, elastography, ultrasound, meat quality

84 Initial investigation of whole-carcass salt-uptake tendencies during immersion chilling. S. Richter*, D. Sabo, and C. Haynes, *Georgia Tech Research Institiue, Atlanta, GA.*

Ice slurry utilizes a brine solution, made in the presently reported study with agricultural sodium chloride feed salt, to keep the slurry in a homogenous state (i.e., reduce ice agglomeration and media separation). This study builds upon a multi-year project examining ice slurry as an alternative chilling medium for poultry processing. This test was developed to determine salt uptake tendencies of whole carcasses (i.e., WOGs) during immersion chilling. High salt uptake and presence in the final meat product has salt-labeling and consumer avoidance-response implications. Whole carcasses were collected post-evisceration and kept at ~30°C temperature. WOGs were chilled for 60 min by either air chill, chilled water (5°C) with 50 ppm peracetic acid (PAA), or 4.5% salinity chilled water (5°C) with 50 ppm PAA. Pre- and post-chilling, all birds were weighed and inner breast meat temperatures were recorded. Post-chilling, 3 sample types were collected per each carcass (breast skin, white meat, and dark meat) from different locations. Carcass samples were removed and analyzed for salt concentration. This initial investigation includes 3 experimental replicates (n = 45 carcasses; total 135 samples). Salt was recovered from the skin and meat samples using a standard organic dry-ash method. The total salt concentration for each sample was determined using an ion chromatograph (IC). Statistical analysis was performed from each sample's IC data, combining sample weight and dilution factor correction. Chloride concentration was calculated as ppm per gram of sample. One-way ANOVA test used to compare chloride concentrations per mass [ppm/g] given groups defined by chilling treatment and carcass skin/meat sampling type, considering mean ± SD (p-value = ≤ 0.05). Initial results show, of all 9 groups (i.e., 3 chilling types * 3 sampling types), 4.5% salinity chilled water skin samples were the only statistically significant group ($P = < 0.0001$) in chloride ppm/g. The 4.5% salinity skin samples (38.1 ppm/g) averaged 14x and 22x higher than chilled water and air chilled skin samples respectively. Results show no statistical significance ($P > 0.05$) between white and dark meat samples between all 3 chilling forms. Results address salt-uptake concerns when ice slurry medium is used for immersion chilling. Initial findings indicate that salt concentrations increase in the skin and does not affect white or dark meat. The skin acts as a barrier that prevents salt penetration into the white and dark meat. Further testing will examine higher salinity immersion chilling to see if salt can penetrate through skin.

Key Words: ice slurry, salt uptake, immersion chilling, whole carcasses

85 Comparison of raw meat quality and protein-gel properties of turkey breast fillets processed by traditional or cold-batter mincing technology. S. Viliani*[1], H. C. Lee[1], P. Singh[2], G. M. Strasburg[3], B. P. Marks[3], and I. Kang[1], *[1]Cal Poly, San Luis Obispo, CA, [2]JBS, Nacogdoches, TX, [3]Michigan State University, East Lansing, MI.*

Cold-batter mincing is an emerging technology that can be used to extract muscle protein in a way to form good protein gels. This study was conducted to evaluate the effects of cold batter mincing on meat quality and protein functionality, using turkey fillets that were chill-boned (CB) or hot-boned (HB) with quarter-sectioning (1/4) and crust-freeze-air-chilling (CFAC) at −12°C (HB-1/4CFAC). For each of 4 replications, 48 toms were raised and processed in a traditional way. After evisceration, the turkeys were hot-boned (HB) or chill-boned (CB) before batter mincing with 1 or 2% salt using the following treatments in 2 Experiments: conventional mincing (4% ice + 16% water) of CB fillets and conventional mincing of HB-1/4CFAC fillets in Experiment I; cold-batter mincing (20% ice) of CB-1/4CFAC fillets and cold-batter mincing of HB-1/4CFAC fillets in Experiment II. During mincing, batters were obtained at 6, 12, and 24 m. Statistical analysis was conducted using 3

factorial design (2 × 2 × 3). Data were pooled due to no interaction ($P > 0.05$) among the factors (boning, salt level, and mincing time) and *t*-test was performed to compare the mincing effects in a same factor. Before mincing, the pH and R-values of turkey fillets in HB-1/4CFAC were higher and lower, respectively, than those in CB fillets. During cold-batter mixing, the initial batter temperatures were at subzero levels (−1.5 to −2.1°C). As mincing proceed, temperatures reached 1.5°C and 14°C at 6 and 12 min, respectively, and ended at 26 to 31°C at 24 min. During traditional mincing, the initial batter temperatures were 3 to 4°C, increased by ~10°C every 6 min, and ended at 32 to 35°C with the higher temperatures seen for 2% salt than 1% salt batter. Dynamic rheological properties of meat batters indicated that the cold-batter mincing showed elevated G' compared with the batters of traditional mincing, regardless of mixing time, indicating that the gel-setting temperature was reduced in the cold-batter mincing, potentially due to more amount of protein extraction and less degree of protein denaturation. After cooking, improved cooking yield and protein functionality were observed in the batters of HB-1/4CFAC fillet, 2% salt, and 6 min mincing than the batters of CB fillet, 1% salt, and over 6 min mincing ($P < 0.05$). These results indicated that HB-1/4CFAC fillets produced superior raw meat quality over the CB fillets, and cold batter mincing of HB-1/4CFAC fillets significantly improved protein functionality compared with the traditional mincing of CB fillets ($P < 0.05$).

Key Words: hot-boning, crust-freeze-air-chilling, cold batter mincing, meat quality, protein functionality

86 Impact of acid treatments on the microbial populations of commercial poultry processing re-use water microcosms. A. Micciche*[1], K. Feye[1], J. Wages[1], C. Knueven[2], and S. Ricke[1], *[1]University of Arkansas, Fayetteville, AR, [2]Jones-Hamilton, Walbridge, OH.*

Sodium bisulfate (SBS) has the potential to be an important antimicrobial in poultry processing facilities due to its application in the produce industry. The re-use of water by poultry plants is increasingly important given the cost associated with the production of potable water and environmental concerns in arid regions. Therefore, it is essential to develop cost-effective strategies to better utilize re-use water and decrease the bacterial load before re-use. This study investigated the microbial populations of re-use water microcosms treated with sodium bisulfate, water treated with peracetic acid (PAA), and untreated processing plant re-use water. Fresh, untreated processing plant re-use water was collected at the end of a poultry processing shift. The water was utilized within one hour of collection and 5 treatment conditions (200 ppm PAA, 1% SBS, 2% SBS, 3% SBS, and untreated) and 3 time points (0, 30, and 60 min) were evaluated. At each time point, aliquots of 50 µL were spread inoculated onto TSA (Tryptic Soy Agar) for aerobic plate counts (APC). The TSA plates were inverted and incubated at 37°C for 24 h and enumerated. There were no statistically significant differences in APC populations among any of the groups and the control with no additive at time point zero. However, all treatments were reduced below the limits of detection after treatment with either SBS (1, 2, and 3%) or 200 ppm PAA compared with the control without an acid additive. There were no differences among acid treatment with SBS (all concentrations) and PAA for the reduction of APC enumerated bacteria on TSA after 30 or 60 min exposure. However, the control poultry processing re-use water retained 4.99e8 to 3.25e10 cfu per microcosm throughout the course of the study. Starting at time point 30 min., the APC microbial populations in the control without additives were significantly greater than the treatment groups ($P < 0.05$). The results of this study indicate that SBS is an equally effective alternative to PAA for decreasing bacterial loads in poultry re-use water.

Key Words: sodium bisulfate, *Salmonella*, re-use water, 16S microbiome, antimicrobial

87 Pulsed UV light as a microbial reduction intervention for boneless/skinless chicken thigh meat. J. Cassar*, E. Mills, J. Campbell, and A. Demirci, *The Pennsylvania State University, University Park, PA.*

Salmonella, *E. coli* and *Campylobacter* are pathogens of concern in poultry processing. A 2012 national data collection survey by the United States Department of Agriculture Food Safety and Inspection Service estimates the prevalence of *Salmonella*, generic *E. coli* and *Campylobacter* on chicken parts to be 24.02%, 62.6% and 21.70%, respectively. Pulsed Ultra Violet (PUV) light is an effective antimicrobial treatment with limited use in the food industry. Research using PUV light has established that it can be a more effective antimicrobial treatment than conventional UV light. The germicidal, UV-C wavelengths fall between 100 – 280nm with the optimum germicidal effect at 254nm. PUV light includes a much broader spectrum, 100 – 1100nm, with 50% of the energy deriving from the UV region. Unlike the continuous, low intensity output of conventional UV light, PUV light is emitted in short bursts of very high intensity light. The current project has investigated application of PUV for destruction of *Salmonella*, *E. coli* and *Campylobacter* on chicken thigh meat. To evaluate PUV light effectiveness, lean and skin surface chicken thighs were inoculated with 6–7 log/cm^2 concentrations of the above mentioned pathogens, independent of one another before exposure to PUV light. Treatment variables included the distance from the quartz window of the PUV light, 8 and 13 cm, and application time, 5, 15, 30, and 45 s. Control samples were not exposed to PUV light. Nine thighs were used for each distance by time treatment combination. An overall 2-way ANOVA with predicator variables, distance and treatment time, showed a significant ($P \leq 0.05$) reduction for each species of microorganism when compared with respective controls. The evaluation of each variable, while holding the other variable constant, showed significant difference due to distance was inconsistent. Microbial reductions for lean surface and skin surface thigh meat were significantly different ($P \leq 0.05$). A Tukey multiple comparison test was used to establish significant differences ($P \leq 0.05$) among treatment times for lean and skin surface thigh averages. Exposure to PUV light for 5, 15, 30 and 45 s resulted in $\log_{10}$ reductions of 1.41^a, 1.63^b, 1.82^b and 2.19^c, respectively for lean surface thighs. The same periods of application time resulted in $\log_{10}$ reductions of 1.05^a, 1.30^b, 1.59^c and 1.86^d, respectively for skin surface thighs. Means for lean and skin surface thighs without a common superscript are significantly different ($P > 0.05$). In conclusion, this study clearly demonstrated the potential of using PUV light as a microbial intervention on chicken meat.

Key Words: chicken, pulsed ultra-violet light, *Salmonella*, *E. coli*, *Campylobacter*

88 The antimicrobial effects of lauric arginate and ethylenediamine tetraacetic acid against *Salmonella* Typhimurium on skinless ground chicken breast. C. Harris*, *University of Florida, Gainesville, FL.*

Antimicrobials are effective at reducing the risk of foodborne illness. Lauric arginate, LAE, is an innovative and non-toxic food preservative with extremely high antimicrobial activity. Lauric arginate is an active ingredient in combating *Salmonella* and combining it with a novel antimicrobial could make it more effective. The experimental approach in this study evaluated the effects of LAE and EDTA against *Salmonella*

Typhimurium on skinless, ground chicken breast. Except for the negative control, the ground chicken meat was inoculated with *Salmonella* Typhimurium (ATCC 13311) and treated with LAE and EDTA. The 9 treatments for the chicken meat included no treatment (meat only negative control), meat plus inoculum (positive control), and meat plus inoculum plus 0.25% EDTA, 0.5% LAE, 1% LAE, 2% LAE, and LAE in combination with 0.25% EDTA. A completely randomized block design with 5 treatments, 5 sampling days, duplicate sample replications per treatment and 2 trials, was used in this study. A total of 180 samples were analyzed. The ground chicken meat was packaged in sterile Whirl-Pak bags, stored at 3 ± 1°C and analyzed on d 0, 1, 3, 5, and 7 for *Salmonella*, total psychrotrophs, and pH. The ground chicken meat treated with the combination of 1% and 2% LAE and EDTA resulted in a significant reduction ($P < 0.05$) in *Salmonella* Typhimurium on all days. There was also a significant reduction ($P < 0.05$) in psychrotrophs on d 3 with the LAE and EDTA combinations. The results indicate that adding the LAE and EDTA to the chicken will decrease the growth of *Salmonella* Typhimurium and psychrotrophs over 7 d.

Key Words: antimicrobial, *Salmonella* Typhimurium, lauric arginate, EDTA

89 Stability of four commercial microbial phytase sources under extreme pelleting conditions. C. Truelock*[1], A. Yoder[1], C. Evans[1], C. Stark[1], S. Dritz[1], J. Wilson[2], N. Ward[2], and C. Paulk[1], *[1]Kansas State University, Manhattan, KS, [2]DSM Nutritional Products, Parsippany, NJ.*

An experiment was designed to evaluate the effects of conditioning temperature and retention time on the stability of 4 commercially available microbial phytases in a starter poultry diet. Treatments were arranged in a 4 × 3 × 2 factorial of phytase source (A, B, C, and D), conditioning temperature (82, 88, and 93°C), and conditioner retention time (30 and 60 s). Diets were formulated to release 0.15% nPP. A total of 5 mash samples were collected from each treatment for analysis of phytase. Diets were pelleted via steam conditioning (245 mm × 1397 mm Wenger twin staff pre-conditioner, Model 150) and using a 30-horsepower pellet mill (1012–2 HD Master Model, California Pellet Mill) with a 4.8 mm × 50.8 mm pellet die. All treatments were replicated on 3 separate days. Retention time was randomized within day and phytase sources were randomized within retention time. During each processing run, pellet samples were collected every 1.5 min and immediately placed in an experimental counter-flow cooler for 10 min. Five cooled pellet samples from each treatment were collected on each day for analysis of phytase. Data were analyzed using the GLIMMIX procedure in SAS, with pelleting run as the experimental unit and day as the blocking factor. There was no evidence for a source × conditioning temperature × retention time interaction for hot pellet temperature or phytase stability. Increasing conditioning temperature from 82 to 93°C increased (quadratic, $P < 0.03$) hot pellet temperature of pellets (94.7, 97.7, and 99.4°C, respectively). There was a phytase source × conditioning temperature interaction ($P = 0.01$) for phytase stability. At conditioning temperatures of 82, 88, and 93°C, phytase stabilities were 33.7, 17.5, and 16.3% for A, 13.0, 8.3, and 9.0% for B, 24.2, 11.2, and 11.8% for C, and 20.7, 11.5, and 9.7% for D, respectively. At conditioning temperatures of 82 and 88°C, A had greater ($P < 0.05$) stability compared with all other sources. At 93°C, there was no evidence of difference between A and C stability, but stability of A was greater ($P < 0.05$) than B and D. Phytase stability of B was less ($P < 0.05$) than that of the other sources when pelleted at 82°C. When pelleted at a conditioning temperature of 93°C, phytase stability was similar between B, C, and D. There was no evidence of difference in phytase stability or hot pellet temperature due to retention time. Microbial phytase stability was reduced by increasing conditioning temperature, although the amount of reduction was dependent on phytase source. Additionally, the maximum phytase stability of 33% observed with equipment used in this study indicates severe consequences of achieving hot pellet temperatures above 94°C.

Key Words: conditioner temperature, enzyme, pelleting, phytase stability, retention time

90 White Leghorn lines divergently selected for high or low antibody response to sheep red blood cells exhibit striking differences in intestinal gene expression following antigen exposure. S. Nolin* and C. Ashwell, *NC State University, Raleigh, NC.*

Gut associated immune functions are incredibly important to animal health, but interactions with systemic immune systems are still not well understood. Two lines of White Leghorns have been divergently selected for over 35 generations for high (HAS) or low (LAS) antibody titer 5 d post injection with low dose sheep red blood cells (SRBC). With > 6 standard deviations difference between mean antibody titers of these lines, they serve as an excellent immunogenetics model. The objective of this study was to use RNA sequencing, to determine intestinal gene expression differences within and between lines, as it changes following SRBC injection. This information will allow for a better understanding of intestinal response to antigens not originating in the gut lumen. Fertile eggs from both lines were obtained from Virginia Polytechnic Institute and State University (Blacksburg, VA) and co-incubated until hatch, after which they were brooded together in mixed cages. Half the birds from each line were injected with a 0.1mL 0.25% SRBC solution at 36 d of age. Six injected birds as well as 6 age-matched non-injected birds from each line were sacrificed and intestinal samples collected for RNA isolation 5 d later. RNAs were extracted via the Qiagen (Hilden, Germany) RNeasy kit, and sent to the NC State University Genomics Sciences Laboratory for library preparation and sequencing on the Illumina HiSeq 2500. RNA sequences were mapped to the Gallus gallus 5.0 assembly and analyzed using CLC Genomics (FDRp < 0.1 due to exploratory nature of the study) followed by Ingenuity Pathways Analysis, both by Qiagen. While both lines share similarities such as activation of genes involved in the oxidative phosphorylation pathway, there were also marked differences in gene expression. Surprisingly, SRBC injection results in over 10 times more genes differentially expressed in LAS than HAS (5000 vs 376). The most highly expresses of the 13 genes exclusively upregulated in HAS when SRBC-injected, is S1PR4, which codes for a receptor of the signaling molecule, sphingosine-1-phosphate (S1P). This particular receptor is virtually uncharacterized in avians, and while not well characterized in mammals, has been found to be expressed almost exclusively in lymphatic cells and is associated with T-cell proliferation, cytokine secretion, and potentially cell motility. Future studies examining S1PR4 and the sphingosine signaling network may offer new insight into physiological changes in the gut in response to systemic stimulation of immune systems. Continued investigation of the many genes differentially expressed in LAS will better characterize phenotypic differences observed between these lines.

Key Words: RNA sequencing, antibody response, gene expression, gut, HAS/LAS

91 Association of the jejunal microbiome and transcriptome with growth in broiler chickens. K. Robinson*[1], Y. Xiao[2], J. Zhao[3], H. Yang[2], and G. Zhang[1], *[1]Oklahoma State University, Stillwater, OK, [2]Zhejiang Academy of Agricultural Sciences, Hangzhou, China, [3]University of Arkansas, Fayetteville, AR.*

Alterations in intestinal microbiota are associated with intestinal and extra-intestinal disorders. In livestock animals, enrichment of specific bacterial taxa has been shown to be associated with growth rate. However, limited information exists on the correlation between intestinal microbiota and growth rate in broiler chickens. To investigate the association of jejunal microbiome and transcriptome with growth in broilers, a total of 90, 38-d-old, healthy broilers of 3 extreme body weights (30 heaviest, 30 medium, and 30 lightest) were selected among 10,000 Ross 308 chicks raised in a commercial production facility under standard management. Thirty mid-jejunal contents from each group of birds were subjected to 16S rRNA gene sequencing, followed by evaluation of microbiota composition using the mothur software package. Five mid-jejunal tissue segments were also collected from each group of birds and subjected to total RNA isolation, followed by RNA sequencing of the transcriptome. Significant differences were determined by one-way ANOVA, analysis of similarity, and linear discriminant analysis effect size (LEfSe), where appropriate. We observed a significant decrease in the evenness of jejunal bacterial community in heavy birds relative to the other 2 groups of birds ($P < 0.01$). Using both the Bray-Curtis and Jaccard indices, we revealed a significant segregation in jejunal microbiota composition among 3 groups of birds ($P < 0.001$). Only *Acidobacteria* group 1 was enriched in the jejunum of heavy birds based on the LEfSe analysis using a LDA score of >3 as the threshold. Another 16 operational taxonomic units (OTUs) were found to be significantly enriched in light birds. Transcriptome analysis revealed 160 differentially expressed genes with a false discovery rate (FDR) of <0.05 and a significant enrichment of 12 gene ontology (GO) terms ($P < 0.05$) between heavy and light birds. It is noted that only the FOXO pathway is significantly enriched in heavy birds, consistent with the fact that the FOXO pathway is known to be critical in glucose metabolism, energy homeostasis, DNA repair, and detoxification of reactive oxygen species. In conclusion, we revealed significant alterations in the microbiota composition and host transcriptional activity in the jejunum of broilers of different growth rates. Manipulation of the bacteria and host genes that are associated with growth could have potential to enhance the performance of chickens.

Key Words: microbiome, microbiota, transcriptome, growth performance, RNA sequencing

92 Genome-wide association study of a commercial egg laying line challenged with Newcastle disease virus. K. Rowland*[1], A. Wolc[2], R. Gallardo[3], T. Kelly[3], H. Zhou[3], J. Dekkers[1], and S. Lamont[1], *[1]Iowa State University, Ames, IA, [2]Hy-Line International, Dallas Center, IA, [3]University of California, Davis, Davis, CA.*

In developing countries, chickens play a vital role in daily life. They provide a constant source of protein through egg production and meat. Newcastle disease, caused by Newcastle disease virus, has been ranked as the most devastating disease for scavenging chickens in Africa and Asia. The loss of these chickens is a devastating loss of dietary protein and buying power for rural households. Improving the genetic resistance of chickens to NDV is a practical target for improvement of poultry production in developing countries. Because response to NDV has a component of genetic control, it can be influenced through selective breeding. Adding genomic information to a breeding program can increase the amount of genetic progress per generation. We hypothesize that many genes regulate NDV resistance in chickens. Our specific objective is to identify genetic markers associated with NDV resistance (reduced viral load, high antibody level). The goal is to apply these markers in a genetic selection program benefiting areas of endemic NDV challenge. In this study, we challenged a commercial egg-laying line with a high titered La Sota vaccine strain of NDV, measured phenotypic responses, collected genotypes (Affymetrix 600k chicken SNP array), and associ-

ated genotypes with phenotypes. Collected phenotypes included viral load at 2 and 6 d post infection, antibody levels pre-challenge and 10 d post infection, and growth rates pre- and post- challenge. Variance components and heritabilities were estimated in AsReml4 and association analyses were performed in an R package (GenABEL). Standard least squares and Student's *t*-tests determined differences in phenotypic responses between time points. Six QTL associated with response to NDV and/or growth were identified. Some were novel and others confirmed previously reported associations with related traits. Additionally, previous RNA-seq analysis provided support for several of the genes located in or near the QTL of the current study. Considering the significant QTL associations and estimates of moderate to high heritability, we provide evidence these NDV response traits can be influenced through selective breeding. The identified QTL can inform breeding decisions for the production of chickens that will be raised in NDV endemic areas. Producing chickens that perform favorably in challenging environments will ultimately increase the supply of quality protein for human consumption.

Key Words: Newcastle disease virus, GWAS, disease challenge, genetic parameters, QTL

93 The role of dietary protein source and genetic background in woody breast transcriptome signatures. S. Peer*, C. Hieke, and G. Athrey, *Texas A&M University, College Station, TX.*

Over the last few years, woody breast (WB) in broilers has been intensely studied using molecular approaches. Transcriptome and pathway analyses have shown alterations in energy metabolism (oxidative stress) and anomalous cell proliferation. The majority of evidence suggests a strong association with broiler growth rate, whereas some studies suggest nutritional interventions that ameliorate WB. The objective of this study was to characterize changes in gene expression in multiple tissue types in response to the source of dietary protein. Furthermore, we also investigated differential gene expression in 2 commercial broiler strains of different genetic backgrounds, both of which presented WB. Replicate broiler flocks (Ross strain) were raised on either an industry standard broiler diet (with soybean meal), or on an energetically equivalent diet with canola meal as the main protein source. Birds were euthanized and sampled at 2-week intervals, over a 9-week study. At the end of the study, birds were scored for WB and tissue samples were collected. We then generated gene expression data from the breast and liver of 64-d old WB+ and WB- birds. For comparison, gene expression data from WB+ and WB- tissue from Cobb strain birds raised on a standard soybean-based diet was also generated. Following isolation of mRNA, the libraries were sequenced on the Illumina platform and analyzed using a custom bioinformatics pipeline. A total of 37 mRNA libraries were sequenced and analyzed. The total transcriptome analysis of the Ross strain revealed only 26 differentially expressed genes (FDR < 0.05) in WB+ compared with WB- tissues. Second, when comparing between diets, we found 10 differentially expressed genes in the pectoralis major muscle of soy fed birds compared with canola fed birds, whereas 46 genes were differentially expressed in the liver. Finally, comparing the Cobb and the Ross strain, we found about 4000 genes differentially expressed in WB+ pectoralis major muscle. Pathway analyses of these data sets confirm previous findings of apoptosis, inflammation, and dysregulation of glycolysis in the breast tissue. In the liver, we found that genes for creatine production are upregulated, whereas the MAPK cascade for cell signaling is downregulated in the liver of WB+ birds. This study confirms our previous results showing limited differentiation between so-called WB+ and WB- scored pectoralis tissue at the molecular level, suggesting that individuals of the same genetic background and flock may not be suitable as a negative control. Furthermore, our study showed the limited differentiation in WB related gene expression between diets, even though the diets elicited significantly different WB severities.

Key Words: woody breast, gene expression, comparative analyses, metabolism, cell communication

94* Comparing rearing parameters and production performance of 1940 random bred control versus commercial strain of Leghorn type pullets grown in the same environment and diet regimen. D. Wall*, N. Anthony, and K. E. Anderson, *North Carolina State University, Raleigh, NC.*

Genetic selection has been utilized to improve the heritable traits of eggs produced, feed conversion and other characteristics provided for continued genetic improvements while strain management tests were conducted to evaluate performance of strains under the same conditions. They provide a mechanism to evaluate the success achieved by selection of those strains involved thus improving the output of future stocks. The aim of this study was to expand the period of selection history by evaluating the pullet growth characteristics of 2 genetic stocks of Leghorns reared under identical conditions. The objective of this study included an assessment of relationship between body weights, feed conversion within the rearing dietary phases of the different strains. 1940 Leghorn Strain (WL40) from the University of Arkansas and a 2016 Commercial Hy-Line W-36 (WLCC) from Hy-Line were housed in the same environment comparing rearing production characteristics from day of hatch until 16 weeks of age. All chicks were hatched at the Prestage Department of Poultry Science at North Carolina State University. All pullets were raised in the same cage rearing facility, 14 birds per brooding cage then with 10 birds per cage. The pullets were weighed bi-weekly, and feed weigh backs were concurrent to determine body growth, feed consumption and utilization. Pullet starter was fed 0–6 weeks, grower from 6 to 12 weeks, and developer from 12 to 16 weeks. Diets were provided ad libitum, and mortality was recorded daily. The experiment was a completely randomized design and all data were statistically analyzed using a one way ANOVA. During the starter, grower and developer feed phase, both strains responded similarly showing an increase in body weights with the 2016 commercial strain exhibiting the heavier weights with no significant differences ($P > 0.05$). Results from this data suggest that selection over the years of layers had no adverse effects on body weights or feed conversion reared in the same environment and current commercial diet thus indicating that selection preferences for desirable traits such as body weights and feed conversion have been successfully implemented for optimal performance.

Key Words: pullet, nutrition, genetics, FCR, body weight

***Corrected abstract (second and third authors added).**

95 In silico verification of microRNA function in the chicken genome. T. Williams*, R. Rohra, and G. Athrey, *Texas A&M University, College Station, TX.*

Micro RNAs, or miRNAs, are small (18–23 base pair) RNA molecules that modulate gene expression by binding directly to a targeted coding region or the untranslated region (UTR) of an RNA molecule. While hundreds of miRNA have been described in the chicken genome, most existing miRNA sequence and their target predictions are based on homology with other species and *ab initio* prediction. However, extensive molecular work is needed to verify and assay their functional significance. The objective of this project was to use in silico approaches

to verify predicted miRNAs and to characterize their functional roles in the chicken genome. Methods: All the predicted miRNA sequences from the chicken genome were extracted from the mirbase database. Each miRNA sequence was queried against the untranslated regions of all protein-coding genes (genome version Galgal4.84), using customized BLAST searches. Results were evaluated for matching quality by the length of the matching sequence, sequence similarity, and mismatch penalties. The similarity of the query to reference sequences were sorted in descending order by E-value and percentage sequence similarity. Only sequences that returned over 90% sequence identity, and fewer than 2bp mismatches, were considered potentially valid. Results: There were approximately 270,000 total miRNA hits to the 3′-UTR from across the chicken genome, but < 1% passed our criteria of > 90% sequence identity. The high sequence identity of these short sequences would be biologically significant for these conserved sequences to target the correct miRNA binding sites. Of those miRNA sequences that were validated this way, few (n < 10) were found to bind to target sites on more than one chromosome. Based on pathway analysis, we identified that miRNAs with multiple targets were active components in major physiological processes including the vitamin metabolism and endocrine receptor pathways. This approach shows the potential utility of in silico methods to filter and validate miRNA predicted from genome data.

Key Words: in silico, microRNA, gene expression, vitamin metabolism, pathway analysis

96 Identification of long noncoding RNAs in chicken divergently selected for leanness. B. Kimathi*, C. Khwatenge, T. Bowden-Taylor, and S. Nahashon, *Tennessee State University, Nashville, TN.*

Long non-coding RNAs (lncRNAs) are non-protein coding transcripts that are more than 200 nucleotides long. They lack an open reading frame of more than 100 amino acids and usually have one or 2 exons. Of all the transcripts from humans, only ~2% code for proteins. Until recently, the non-coding transcripts were thought of as "junk DNA." LncRNAs play crucial roles in transcriptional regulation of biological processes. The objective of this study was to identify and characterize lncRNAs from adipose tissue of chickens divergently selected for leanness. 59,884,218 and 26,240,352 paired-end sequence reads generated using Illumina platform using total RNA from adipose tissue of fat line (FL) and lean line (LL) respectively were downloaded from National Centre for Biotechnology Information's (NCBI) SRA website were used. FastQc was used to analyze the quality of the reads and the high-quality reads were retained for further processing. Cufflinks software was used to assemble the transcripts which were then submitted to Flexible Extraction of long non-coding RNAs (Feelnc) software to distinguish non-coding from the coding transcripts using a user-generated coding potential score of 0.523. The filter module in Feelnc was used to remove all the protein-coding transcripts by comparing the transcripts to the nonredundant protein database, the codpot module was used discriminate the long noncoding transcripts based on their size and protein-coding potential. We predicted a total of 1532 putative lncRNAs from adipose tissue of the FL chicken. Of these, 1023 were further classified as genic while 509 were classified as intergenic. From the LL chicken, a total of 1155 putative lncRNAs were predicted with 695 classified as genic and 460 as intergenic. The project adds to the database of chicken lncRNAs. Comparison between the expression of lncRNAs in the FL and LL chickens will help in highlighting the specific lncRNAs that may have regulatory roles in adipogenesis. These will provide targets for further analysis into their mode of action.

Key Words: long noncoding RNA, adipose, broiler, adipogenesis

Student Competition: Extension and Instruction

97 Assessing educational needs and practices of poultry fair participants relative to biosecurity and avian infuenza. M. Olson*, H. Martin, C. Cardona, and S. Noll, *University of Minnesota, Saint Paul, MN.*

After the 2015 outbreak of highly pathogenic avian influenza in Minnesota, the potential for transmission at fairs raised the question of awareness of contestants about biosecurity and avian influenza. The objective was to identify key biosecurity practices of poultry contestants before and after the fair and how they rated their knowledge of avian influenza. Information was collected with face-to-face surveys at 2 Minnesota county fairs and then at the Minnesota State Fair in 2017. A short survey with 7 questions was used due to time limitations for contestants during check-in and was administered to 4-H youth and accompanying adults at the fair facilities. Because of the small number of surveys collected from the 2 county fairs (14, 18), responses were combined with those of the state fair (161) for a total of 193 completed surveys. The respective percentage of respondents that were adults, youth between 12 to 17 years of age, and youth less than 12 years of age was 29, 65 and 5%. Over one-half the contestants were exhibiting chickens (57%) followed by waterfowl (20%). When respondents were asked to rate their knowledge about avian influenza, 63% indicated moderate with 18% indicating little knowledge. Many respondents were using one or more of the following biosecurity practices at home: quarantine (53.9%); controlling access of people and equipment (37.8%); and, using cleaning and disinfection practices (66.1%). About 23% of participants used a combination of all 3 practices. A small percentage of respondents (11.4%) indicated not practicing biosecurity. After the fair, 48% of the respondents indicated using quarantine for poultry brought back home. An open-ended question was asked so that respondents could indicate what types of information they need. The comments were categorized into areas of disease prevention, spread/risk, clinical signs/detection, treatment, and communications in regard to disease outbreaks. Based on the survey results, there is a need for on-going poultry health education including avian influenza and biosecurity. To improve effectiveness of biosecurity practices, combinations should be considered rather than reliance on a certain practice. Funding for this project was provided by the Minnesota Department of Agriculture through the University of Minnesota Response to Avian Influenza Project.

Key Words: biosecurity, fair, exhibition, 4-H, youth

Student Competition: Animal Well-Being and Behavior II

98 Effects of stocking density on the perching behavior of 3 strains of pullets. L. Jensen* and T. Widowski, *University of Guelph, Guelph, ON, Canada*

Moving forward, more pullets will be housed in complex environments to fill the demand for laying hens that can cope with multi-tier systems. One of the main features of new rearing systems is perch access from d 1 of life. However, little is known about how much perch space pullets need or if stocking density (SD) impacts perching behavior. The objective of our study was to investigate perch use in 3 commercial strains of pullets (LSL-lite [LSL], Lohmann Brown [LBR], and Dekalb White [DK]) housed at 4 SDs in closed rearing cages (Combi Pullet: Farmer Automatic) furnished with 3 length-wise perches and a raised platform, all differing in height. We hypothesized that a greater proportion of pullets would perch at higher SDs and that the white strains would perch more than the brown strain. Floor space allowance (cm^2/bird including platform) and number of birds per cage were 246.5 cm^2, 91 birds; 270.3 cm^2, 83 birds; 298.7 cm^2, 75 birds; and 334.8 cm^2, 67 birds. Perch space per bird ranged from 7.8 cm to 10.7 cm at the highest and lowest densities, respectively. There were 3–4 replicate cages for each SD and strain (n = 45) which were balanced across 2 rooms. One or 2 observers counted the number of birds on each perch and the platform one d per wk from 1 to 15 wk of age. Four live observations were performed during the day approximately every 3 h starting when lights came on. The effects of strain and SD on percentage and mean number of birds perching were analyzed using the GLIMMIX procedure in SAS University Edition. Throughout the 15 wks, LSLs perched the most with an average of 25% ± 0.5 of birds using all of the 3 perches and LBRs the least (15% ± 0.3) ($P < 0.001$). For all strains, the most rapid increase in perching occurred between 2 and 4 wk of age. The white strains continued to increase perch use gradually until 15 wks whereas perch use decreased after 7 wk of age for the brown strain. SD did not affect the percentage of birds perching (21.4% ± 0.2; $P = 0.505$) but did significantly affect the mean number of birds perching ($P < 0.001$) with the most birds perching at the highest density (22.4 ± 0.5) compared with the lowest density (17.2 ± 0.4). The maximum number of birds observed on a single perch at 15 wk of age for LSL, DK, and LBR were 19, 19, and 15, respectively. This means the smaller white strains used a minimum of 12.5 cm of perch space per bird whereas the larger brown strain used 15.8 cm. In summary, a greater number of birds perched at higher SDs, but this did not translate to a greater overall proportion of birds perching so SD may not motivate perching. Strain differences were apparent, and strains may have different perch space needs based on overall perch use and body size.

Key Words: pullet, perching, stocking density, strain

99 The impact of infrared beak treatment on turkey behavior and heterophil to lympocyte ratios. T. Fiss*[1], S. Gomis[2], H. Classen[1], R. Dickinson[2], and K. Schwean-Lardner[1], *[1]University of Saskatchewan, Saskatoon, SK, Canada, [2]Department of Veterinary Pathology, University of Saskatchewan, Saskatoon, SK, Canada.*

Removing the tip of the top beak with infrared beak treatment (IRBT) is used to reduce damage from injurious pecking. The objective of this work was to examine the impact of IRBT on the behavioral and stress responses of turkeys. Turkeys (toms (27/pen) or hens (38/pen), IRBT or untreated (UT), with 4 pens per treatment × sex) were housed in a 2 × 2 factorial arrangement and reared for 12 wk at a predicted final housing density of 32 kg/m^2. Blood samples were taken from 20 birds/treatment on 1, 5, 10, 15 and 20 d of age. Slide smears were made and stained with a Hema 3 kit. Leukocytes (100) were identified and heterophil to lymphocyte ratio (H:L) was determined. Birds from 2 replicate pens/treatment were videotaped (HFR700 camcorders, 24h) on d 1, 6 and 8, and then every pen was videotaped (A-CBVD36PI infrared bullet cameras, 24h) at 19, 60 and 81 d of age. Behaviors were classified using scan sampling every 15 min. Log+1 transformations were performed if the data were not normally distributed. Data were analyzed as a RCBD (SAS 9.4) using Proc Mixed. Significance was declared at $P \leq 0.05$. IRBT decreased H:L ratios on d20 but treatment had no impact before that. There were significant interactive effects on behavior. On d1 UT toms walked more than IRBT toms, but neither differed significantly from the hens. On d6 UT toms and IRBT hens spent a smaller percent of time at the feeder. On d81 UT toms spent a greater percent of time at the drinker than other birds, IRBT toms preened more than other birds, and IRBT toms pecked the environment more than IRBT hens. Other behaviors were significantly impacted by the main effects. On d1 IRBT birds were more inactive and environmental pecked more than UT birds. On d6 IRBT birds stood more, were less active, and foraged less than UT birds. On d8 IRBT birds walked more and spent a lower percent of time at the feeder. On d19 and 81 IRBT birds a spent a greater percent of time at the feeder. On d60 treatment had no effect. Toms on d1 spent a lower percent of time at the drinker than hens. On d6 percent of time inactive was higher for toms and lower for standing, at the drinker, and pecking the environment than hens. On d8 toms walked and foraged more than hens. On d8, 19 and 81 toms were less active, performed more aggressive behaviors, and stood less than hens. On d60 toms were more inactive, and spent a lower percent of time at the feeder and standing than hens. To conclude, minor changes in behavior were noted when IRBT was applied. However, the H:L ratios suggest stress is reduced with IRBT at 20d of age.

Key Words: stress, environmental pecking, activity

100 Effect of LED lighting on turkey hen feathering and performance. B. Bartz*, J. Grimes, and C. Nestor, *North Carolina State University, Raleigh, NC.*

Turkey hens of the same strain were reared under light emitting diode (LED) conditions with a range in Kelvin (K) temperature and 2 d lengths, 12 h (S) and 18 h (L), until 5 wks of age. Light treatments (TRT) included 5,000K (5K), and 5,000K + Far-Red LED light enhancement at 629 nm (FR). At 5 wks, half of the birds were moved to a natural lit and ventilated facility where they received either natural day length (NAT), or a 5,000K, 18 h blocked day length in combination with natural light conditions (18BLK). In all cases, 8 – 10 footcandle (Fc) light intensity was achieved between LED light TRT and natural Fc intensity was used for the NAT and 18BLK TRT. Performance data were analyzed at 5, 9, and 14 wks of age. Data were analyzed using JMP 13.2 as a one-way ANOVA and LSMeans were separated using Tukey's HSD with a significance value of $P \leq 0.05$. Brooding TRT was used as a covariate in the model for data following 5 wks of age to correct for variation between TRT during the brooding phase. There were no differences in feed consumption (FCN) between TRT at 5 wks of age (SEM = 0.04, $P = 0.0444$), however, there were significant differences in body weight gain (BWG) and feed conversion ratio (FCR) between lighting TRT (BWG: NAT = 1.24^b, 18BLK = 1.33^a, S5K = 1.30^{ab}, L5K = 1.29^{ab}, FRS = 1.23^b, FRL = 1.33^a; SEM = 0.019, $P = 0.0078$ and FCR: NAT =

1.64[a], 18BLK = 1.59[ab], S5K = 1.51[b], L5K = 1.56[ab], FRS = 1.58[ab], FRL = 1.60[ab]; SEM = 0.017, $P = 0.0176$, respectfully). Period performance data from 5 – 9 wks, the first half of the grow-out phase, this trend was reversed in which differences were measured in FCN between TRT (NAT = 5.36[b], 18BLK = 5.41[b], S5K = 5.19[b], L5K = 5.93[a], FRS = 5.60[ab], FRL = 5.66[ab]; SEM = 0.106, $P = 0.0020$) and no differences observed between TRT with regards to BWG and FCR (BWG: SEM = 0.083, $P = 0.0913$ and FCR: SEM = 0.054, $P = 0.295$, respectfully). From 9 – 14 wks of age, or the latter half of the grow-out phase, there were no differences between TRT with respect to FCN, BWG, or FCR (FCN: SEM = 0.325, $P = 0.0619$, BWG: SEM = 0.231, $P = 0.1458$, and FCR: SEM = 0.076, $P = 0.9982$, respectfully). Performance from 5 to 14 wks indicated no differences in FCN or FCR throughout the grow-out phase (FCN: SEM = 0.310, $P = 0.3733$ and FCR: SEM = 0.033, $P = 0.5566$, respectfully). However, there was a significant difference in BWG between TRT during the grow-out phase (NAT = 8.55[a], 18BLK = 8.23[ab], S5K = 8.43[ab], L5K = 8.19[ab], FRS = 8.30[ab], FRL = 7.99[b]; SEM = 0.151, $P = 0.0218$). Given these results, there appears to be a biological response to changes in lighting parameters with regards to bird performance, however, additional testing will need to be completed to determine the significance of these differences.

Key Words: LED lighting, kelvin temperature, brooding, growout, turkey hen

101 Baseline cecal motility and feather pecking in laying hens. J. Krumma*[1], N. van Staaveren[1], P. Forsythe[2], J. Kjaer[3], Y. Kwon[1], Y.-K. Mao[2], C. West[2], W. Kunze[2], and A. Harlander-Matauschek[1], *[1]University of Guelph, Guelph, ON, Canada, [2]McMaster University, Hamilton, ON, Canada, [3]Friedrich-Loeffler-Institut, Celle, Germany.*

Human patients suffering from gastrointestinal disorders also show an increased number of behavioral disorders. One of the most important behavioral problems in laying hens, feather pecking (FP) continues to reduce bird welfare and increase economic losses. While the underlying causes of FP are multifactorial in nature, previous work has demonstrated an association with feather eating. Gut feed passage time is known to be accelerated in High Feather Pecking (HFP) animals, as these birds ingest feathers that act as a form of insoluble fiber. However, it is unknown whether feed passage time differences are achieved by increased motility to propel digestive contents or by feathers per se. In a first step to approaching this question, we hypothesized that these differences in feed passage time might be caused by differences in ceca smooth muscle contraction, as the ceca plays an important role in insoluble fiber digestion and triggering propulsive peristalsis. As of yet, no study has analyzed gut motility in an ex-vivo model in FP laying hens. Thus, we compared ceca motility in an organ bath perfusion system in HFP and unselected control (C) birds. Twenty-five birds at 64 weeks of age were selected on the basis of genotype (11 C and 14 HFP). Birds were euthanized via cervical dislocation and cecal segments excised and placed in the well of an tissue bath perfusion system filled with an oxygenated buffer solution. Silicone tubing was used to cannulate the cranial and caudal ends of the tissue to allow for flow of oxygenated physiological saline buffer solution through the lumen of the tissue. Contractions of cecal segments were recorded using a JVC camcorder and recordings were analyzed using specialized ImageJ software with a specific plug-in (DMaple©) to generate spatiotemporal maps. These maps depict contractility of a tissue over time and enable the measurement of the frequency, velocity, and amplitude of propulsive peristalsis. These measurements have been validated by numerous other studies measuring intestinal motility in other species. Data were analyzed using a generalized linear mixed model (GLIMMIX) in SAS version 9.4. No significant differences in frequency, velocity or amplitude of cecal contractions between control and HFP birds were found ($P > 0.05$). Future work will focus on determining whether differences may be found when the phenotypic profile of laying hens is taken into account.

Key Words: laying hen, feather pecking, genetic line, motility, ex vivo

102 The effect of gradient lighting on commercial broiler chicken feeding and drinking behavior. D. Aldridge*, C. Scanes, C. Hayes, and M. Kidd, *University of Arkansas, Fayetteville, AR.*

The objective of this study was to compare feeding behavior parameters of commercial broilers reared using conventional over-head lighting to those in a light intensity gradient environment. It was hypothesized that there would be no differences in feeding behavior in broilers reared under conventional or a gradient lighting environments. Cobb 700 broilers were housed in 16 (6.10 × 1.52 m) pens in 2 commercial broiler production houses. Broilers were reared under 2 different lighting treatments beginning at placement: conventional lighting: overhead LED and CFL bulbs (overdrive) providing > 40 lx (d1 to d14) and overhead LEDs only (d14 and older) at 5 lx; Gradient: > 100 lx from a LED light fixture (AviLighting) attached to a feed line to 40 lx furthest from the feed line from over-head LEDs and CFLs from d1 to d14 and a gradient from 111 lx at the feeder to 2 lx furthest from feeders for the remainder of the grow out from the feed line lighting only. Data on behavior was collected using video recording of 16 pens (8 pens of each treatment) during the first, last, and a random hours during d15, d21, d35 and d40. The number of birds actively feeding, actively drinking, standing near feeders, standing near water lines, setting near feeders, and setting near water lines were recorded every 15 min during the sample times. Final body weights were also collected on d 41. Data were analyzed by one way ANOVA with differences were determined using Tukeys HSD test. Broilers reared under the gradient lighting were heavier ($P < 0.05$; BW = 2.67 kg) than those reared using conventional lighting (BW = 2.54 kg). However no differences in feeding behavior were observed for any of the sample days. Birds reared with gradient lighting had increased drinking behavior at day35 over birds reared using conventional lighting. More ($P < 0.05$) broilers were observed to be sitting near feed on d 15 in the feeder line lighting house (8.34) than the conventional (5.47). However the inverse was observed on d 35 and 40. The number of birds setting near feed were greater in the conventional house on (d 35–12.4 and d 40–13.9) than observed in the feeder line lighting house (9.79 and 9.49). The observations collected during this investigation provide multiple insights to the influence of lighting on commercial broiler feeding behavior. It was observed that the use of feeder line lights increased the number of broilers near feed during brooding (till d 21). Second, the provision high intensity (>100 lx) near the feeder reduced the number of broilers setting near the feeders later during the growth period and potentially allowing other broilers to access the feed more easily.

Key Words: lighting, gradient, feeding, behavior

103 Impact of social and non-social stress during adolescence on feather cover of laying hens. C. Mindus*[1], N. van Staaveren[1], H. Champagne[1], P. Forsythe[2], W. Kunze[2], J. Kjaer[3], and A. Harlander-Matauschek[1], *[1]University of Guelph, Guelph, ON, Canada, [2]McMaster University, Hamilton, ON, Canada, [3]Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Celle, Germany.*

Feather pecking (FP) is a behavior in which individual laying hens peck repetitively at the plumage of conspecifics, causing poor feather

cover. Although FP can be caused by many factors, the development of its most severe form appears to be enhanced by difficulty coping with social and non-social stress. Laying hens are often subjected to stress, including adverse social life events such as constant mixing and limited access environmental enrichment when birds are housed in large flocks. These events predispose animals to perform anti-social behaviors. The aim of the present study was to investigate how Unpredictable, Repeated Stressors (URS) during adolescence affect birds' interest in and reactions toward conspecifics. We hypothesized that birds subjected to URS would express more anti-social behaviors toward each other, resulting in poorer feather cover and more head and neck injuries. White Leghorn laying hens (n = 86) were individually tagged and systematically assigned to 6 stressed (S) and 6 non-stressed (NS) groups (7 birds per group, 19 weeks of age). Each group was housed in an enriched floor pen under commercial management conditions. Pens were video recorded for 4 weeks as a baseline to identify aggressive behavioral patterns of individual birds. The 6 S groups were exposed to social (i.e., mixing with unfamiliar birds, manual restrain for 5 min, holding in a transport crate in a crouched position for 1 h) and non-social (i.e., introduction to a novel environment, removal of nest boxes or perches) URS while the NS groups were left undisturbed. Behaviors were recorded after exposure to these URS for both the S and NS groups. All behavioral recordings were performed by the same blinded observer. Additionally, feather cover and injuries to the comb/head were scored on a binary scale and were collected both before and after URS for all birds. A generalized linear mixed model (Proc Glimmix) was used to analyze the data (SAS V9.4, SAS Institute, Cary NC). Variance of aggressive interactions, feather cover and injury scores were partitioned into the fixed effect stress using the most appropriate distribution. Baseline behavioral pattern and body weight were used as covariates. Our results revealed that aggressive interactions were significantly increased by the stress treatment (P = 0.0091). S birds also had significantly poorer feather cover and more injuries (P = 0.0057 and P = 0.03, respectively). These results show that a combination of social and non-social stressors during adolescence trigger aggressive interactions and negatively impact the feather cover of adult laying hens.

Key Words: laying hen, repeated unpredictable stressors, feather cover, aggressive interaction, anti-social behavior

104 Hazardous pathways resulting in falls within multi-tier housing systems for laying hens. N. Mackie*[1], A. Stratmann[2], S. Buijs[3], J. Tarlton[1], and M. Toscano[3], *[1]University of Bristol, Bristol, United Kingdom, [2]Universitat Bern, Bern, Switzerland, [3]Agri-food and Bioscience Institute, Belfast, United Kingdom.*

Multi-tier housing systems for laying hens are increasingly popular within Europe and the US because of the availability of resources and freedom of movement they provide. However, the complexity and/or height of the internal housing can lead to falls and collisions. These falls and collisions are thought to cause the increased prevalence of keel bone damage that is reported for multi-tier housing systems compared with single tier and cage systems. The aim of this study was to identify hazardous areas and pathways within a single multi-tier housing system that resulted in high proportions of falls compared to controlled movements. A semi-commercial multi-tier unit was used consisting of 20 pens containing 200 birds each, with 8 pens used throughout the study. Tri-axial accelerometers were attached to the keel bones of 7-8 birds per pen to measure activity. The study covered 2 flocks ranging from 19 to 36 weeks of age and 51 to 61 weeks of age. In total, approximately 20 weeks of data were collected with data being collected in 2 pens each week. The behavior of each focal bird was observed using video observation. Data were analyzed using a generalized linear mixed model. Proportion of falls (Y/N) was used as a response variable with bird nested within pen as a random factor and movement path (i.e. from where the movement started and finished), time of day, feather cover, keel bone fracture status, foot pad lesions (dermatitis and bumble foot), body weight and flock used as fixed factors. Multiple comparisons were tested using Bonferroni corrections. Movement path affected the proportion of falls ($P < 0.0001$). Many differences were noted, with only a small selection of differences given here. Movements upwards between a lower perch and a middle perch (75° angle and 0.65m in height, n = 114) resulted in fewer falls (25.0% ± 6.2%) compared to movements upwards from the nest box to a middle perch (50° angle and 0.37m in height, n = 70) (77.8% ± 6.0%) ($P < 0.0001$). Movements downwards from the top tier to a middle perch (60° angle and 0.30m in height, n = 126) resulted in a higher proportion of falls (75.4% ± 4.2%) compared to downward movements from a middle perch to the litter (height 1.9 m) (39.3% ± 4.9%, n = 214). There was an effect of time of day on the proportion of falls ($P < 0.0001$) with more falls occurring during the night (with the majority occurring within the first hour after lights off) followed by dusk, dawn and day, respectively. The data suggest that different areas within a standard multi-tier housing system and times of day result in different proportions of falls and thus it is important to take these data into account when customizing welfare friendly housing systems for laying hens.

Key Words: multi-tier system, falls, movement, behavior, accelerometer

105 Evaluating five carbon dioxide induction methods for the gaseous euthanasia of day-old broiler chicks testing time to aversion, insensibility, and death. B. Baker*[1], S. Torrey[2], P. Turner[2], T. Widowski[2], T. Knezacek[1], J. Fricke[1], S. Gomis[1], H. Classen[1], T. Crowe[1], and K. Schwean-Lardner[1], *[1]University of Saskatchewan, Saskatoon, SK, Canada, [2]University of Guelph, Guelph, ON, Canada.*

To evaluate the efficacy of 5 CO_2 euthanasia induction techniques at inducing aversion, insensibility and death, cull broiler chicks (n = 110) were euthanized on day of hatch by immersion into 100% CO_2 (IM), or gradual induction (GI) to 100% CO_2 at flowrates of 3, 6, 9 or 12 L per minute (LPM). Two chicks per replicate (11 reps/trt; RCBD) were placed into a Euthanex chamber (42.5L) that was either prefilled with 100% CO_2,or contained ambient air and gradually filled with CO_2. Live focal sampling (2 observers) and video recording were used to measure chicks' time to behavioral responses: head shaking (HS) and gasping (GS) as indicators of aversion; loss of posture (LOP) as an indicator of insensibility; and cessation of rhythmic breathing (RB) and movement (COM), indicating death. CO_2 concentration at chick level was recorded via a CO_2 sensor. Behavioral and CO_2 concentration data were (log+1) transformed before statistical analyses. One-way ANOVA with induction trt as main effect, block as random effect, and chamber as experimental unit was performed (PROC MIXED, SAS 9.4), with means separation via Tukey-Kramer tests. Regression analyses (PROC REG, RSREG) determined the effect of GI flowrate. Differences were considered significant when $P < 0.05$. All behavioral indicators showed that between trts (shown in order of IM, 3, 6, 9 and 12), aversion (GS: 3, 24, 22, 19, 16s; HS: 1, 16, 17, 12, 10s), insensibility (LOP: 9, 80, 62, 59, 51s) and death (RB: 18, 441, 295, 240, 190s; COM: 56, 554, 388, 317, 232s) occurred earliest with IM compared with GI. Time between first performance of HS and LOP was shorter for IM than any GI trts, with the longest time noted at 3 LPM (64, 45, 47, 41 and 8s for 3, 6, 9, 12 LPM & IM, respectively). Increasing flowrate (3, 6, 9 and 12 LPM)

showed a linear decrease in time to first performance of GS (24, 22, 19, 16s), HS (16, 17, 12, 10s), and LOP (80, 62, 59, 51s), respectively. Time to RB decreased quadratically with increasing flowrate (441, 295, 240, 190s for 3, 6, 9 and 12 LPM, respectively), while COM decreased linearly (554, 388, 317, 232s for 3, 6, 9 and 12 LPM, respectively). Within GI, COM was the only behavior that differed as a response to CO_2 concentration at time of performance, with the concentration being lower with 3 than 9 LPM (69 v 77% CO_2). Overall, time between aversion and insensibility was shortest for IM, and behavioral indicators of insensibility and death occurred earliest with IM. For GI, insensibility and death occurred quicker with higher flowrates then than lower flowrates. Aversion behaviors occurred with all trts and aversive exposure time was longest with the low flowrate.

Key Words: immersion, welfare, cull, disposal, induction

106 Prevalence of keel bone damage in laying hens can be influenced by using ramps in pullet rearing and laying hen aviaries. S. MacLachlan*[1], A. Ali[1], A. Stratmann[2], M. Toscano[2], and J. Siegford[1], *[1]Michigan State University, East Lansing, MI, [2]University of Bern, VPH-Institute, Zollikofen, Switzerland.*

In recent years cage-free laying hen housing styles, such as the aviary, have gained popularity because they allow for more species-typical behavior. Multi-tiered aviary structures give room for the hens to roost, dust bathe, perch, and are thought to increase hens overall welfare. However, hens face challenges in aviaries, such as mastering the ability to navigate vertical space. When using the resources provided by the aviary hens are exposed to risks of falling or colliding with conspecifics or structures in the system, which can lead to bone damage including of the keel. Our main objective was to investigate whether provision of ramps during rearing would reduce subsequent incidence of keel damage, and whether continuing provision of ramps in laying housing would further reduce the incidence of keel damage. 4,800 LSL pullets were raised in 2 treatments: 4 pens/600 birds were raised with ramps to aid in movement between tiers of the aviary (RR, reared with ramp) and 4 pens/600 birds were raised without ramps (RN, reared no ramps). At 17 weeks all birds were moved to the laying facility, in which there were 16 aviary pens with 225 birds/pen. Half the laying facility pens (n = 8) were supplemented with ramps (LR) and the other half were not (LN). Within each laying treatment, 4 pens were populated with RR pullets and 4 pens were populated with RN pullets. From each pen, 15 focal hens were selected and keel bones x-rayed at 21 and 36 weeks. These images were scored on a 6-point scale to assess the amount of bone affected by damage (0-little to no damage, 5-severly damaged). Data were analyzed using GLMM (multinomial) with rearing and laying treatments as main effects and pen as a random effect (α set at 0.05) with R software. At wk 21, hens that were reared with ramps and moved into a laying facility enriched with ramps (RR-LR), showed the lowest level of keel damage (Score 0: 98% of hens), while those reared with ramps and moved into ramp free laying pens (RR-LN) showed the most keel damage (Score 0: 88% of hens; $P = 0.04$). At wk 36, again, hens with RR-LR treatment showed the lowest level of keel damage, with 48% of hens scoring 0, versus 38% in RN-LN, 36%, and 35% in RR-LN and RN-LR respectively (All $P < 0.05$). No ramp treatment completely eliminated keel damage in these young hens; however, providing ramps in both pullet and laying housing notably reduced keel damage in laying hens when compared with ramps in either pullet or laying housing or to no ramps at all.

Key Words: rearing aviary, ramp, bone strength, laying hen, pullet

107 Behavior of market turkey toms in a partial slotted flooring system. E. Theis*, J. Brannon, K. Janni, and S. Noll, *University of Minnesota, Saint Paul, MN.*

Meat type poultry have traditionally been reared on floors covered with bedding because hard surfaces can lead to leg problems and carcass (breast) defects. Development of breast defects is suspected to be due to the poultry resting on the hard surface. A new turkey production system is being explored which is a combination of floor area composed of slotted flooring (SF) and bedding. The main objective of the research is to determine how turkeys utilize the SF area and if behavior differs among flooring materials. Male market toms were raised in pens with 25% of the floor area replaced with SF from 5 to 18 wks of age. Six flooring treatments included Control (C, 100% bedding) and 5 treatments with different types of commercially available SF. The 5 flooring materials (slat opening size and shape) were: Double L Classic Red Rooster (1.9x6.4 cm rectangular); SW Ag Plastics Dura-Slat STO (2.8 cm square); SW Ag Plastics Dura-Slat ST (2.8 cm square); Tenderfoot (2.2x5.5 cm rectangular); and Tenderfoot (2.5 cm square). One galvanized hanging feeder and 2 Plasson bell waterers were located in each pen and were placed over the SF area where present. Fresh wood shavings bedding was used in all pens. Treatments were randomly allocated to 2 replicate pens with 50 toms (Large White, Hybrid Converter) per pen. The study was conducted in the fall season. Cameras (xmarto 8-Channel 960p HD Wireless Security Camera System) were used to record behavior on a 24 h basis at 10 and 18 wk of age. Behavior scans were conducted at 5 times during the day: 0500, 0845, 0915, 1715, and 1745 h. The times were selected to observe maximal frequency of resting (RE), feeding (FE), and drinking (DE) events. Recordings were done on Sundays to minimize interference from worker and farm activity disturbances. Data was analyzed using the proportion of total birds in the pen displaying RE, FE and DE behaviors on SF. Analysis of variance was conducted with the model including flooring treatment (F) and time (T) at sampling and their interaction (F*T). The proportional data was transformed to $\log(x+1)$ for statistical analysis. Slotted flooring type did not affect behavior (RE, FE, DE) on the SF or percentage of birds on SF at either age ($P > 0.05$). A contrast test of flooring type vs control indicated no differences in FE and DE ($P > 0.05$). Percentage of birds resting on SF was greatest at 0500 h, averaging 9%. The RE mean for the experiment was 2.5%. In conclusion, male turkeys used SF on a limited basis and locating the feeders and waterers over SF did not restrict FE and DE behavior as compared with the control pens with 100% bedding.

Key Words: turkey, slotted flooring, behavior

108 The effect of maternal age, rearing and housing environments on yolk testosterone and fear-related behavior of leghorn chicks. L. Cooley* and T. Widowski, *University of Guelph, Guelph, ON, Canada.*

Commercial strain leghorn breeders are reared and housed in a variety of housing systems where they are exposed to various physical and psychological stressors. Maternal experience can affect offspring phenotype through epigenetic changes that can occur during gametogenesis, when breeding stock are adolescents, and by changes in egg composition when breeders are in lay. Previous research on wild birds suggests an environmental influence on the physiology of the mother and highlights the influence of yolk androgens on hormone-mediated changes to offspring phenotype. The objectives were to investigate influences of maternal rearing and housing environments and maternal age on yolk testosterone (T) concentration and fear-related behavior of progeny in

commercial leghorns. Two cohorts of LSL-Lite leghorns were reared in aviary (AV) or conventional battery cages (CC) from d 1, then housed in AV (n = 2), CC (n = 12) or large furnished cages (FC) (n = 12) from 16 wks of age (woa) resulting in 5 rearing × housing treatments: Trt1 (AV × AV), Trt2 (CC × CC), Trt3 (AV × CC), Trt4 (CC × FC), and Trt5 (AV × FC). Hens from each trt (n = 48/trt/cohort) were inseminated with pooled semen at each of 3 ages: Young (25 woa), Ideal (44 woa) and Old (68 woa). A sample of eggs collected 2 d before insemination was analyzed for yolk T. Fertile eggs from all trt groups within a cohort were stored, incubated and hatched together. Each cohort had 4 replicate groups of progeny per trt (7 males and 7 females), identically reared in furnished floor pens. At 21, 35, and 70 d of age each group of progeny was exposed to a Novel Object (NO) for 2 min. At 63 d of age, 2 males and 2 females from each replicate were individually tested for Tonic Immobility (TI) for 10 min. Repeated GLIMMIX procedures (SAS 9.4) (cohort = random) were used to test for effects of trt and maternal age. Contrasts were used to compare effects of maternal rearing and adult housing environments. Young hens deposited more T in yolks than Old hens ($P = 0.0260$). Yolk T decreased with hen age: Young = 1.46 ng/g yolk, Ideal = 1.31 ng/g yolk, Old = 1.06 ng/g yolk. Fear response to the NO decreased ($P \leq 0.0395$) with both maternal age of the breeder hens and with progeny age ($P < 0.0001$). Pullets from all maternal ages and treatments required a greater ($P \leq 0.0003$) number of induction attempts, had a shorter ($P < 0.0001$) latency of TI; but with a higher ($P < 0.0141$) number of distress vocalizations than cockerels. Progeny from breeder hens reared in AV had a shorter ($P = 0.0045$) latency of TI than those whose mothers were reared in CC. Rearing experience as well as age of hens affected egg traits and progeny.

Key Words: maternal experience, progeny, fear, yolk hormones

109 Modeling bone development characteristics of layers. C. Chen*, S. Aggrey, and W. Kim, *University of Georgia, Athens, GA*.

Understanding the layer bone model parameters and their relationships provides a sounds basis for creating the strategies to optimize bone development and alleviate osteoporosis during laying period. A study was conducted to explore the bone development pattern of modern high egg producing laying hens. A total of 480 1-d-old Hy-line W36 pullets were raised until 21 wk based on Hy-line W36 guide. From wk 0 to 21, 10 birds per week were weighed and femurs were taken for Micro-computer tomography(Micro-CT) analysis. Segmented linear and exponential models were fitted to the body weight(BW), total bone tissue volume(TV), calcified bone volume (BV), bone mineral density(BMD) and bone mineral content (BMC) data by using NLIN procedure. From results, The BW and TV fitted to logistic exponential model ($P < 0.001$, $R^2 > 0.979$). BW and TV increased exponentially by 0.256 g/wk and 0.320 mm^3/wk, respectively. BV and BMC fitted to linear model from wk0–16 and exponential model from wk17–21 ($P < 0.001$, $R^2 > 0.970$). BV had a growth rate of 105.1 mm^3/wk up to 17wks and followed by an exponential growth by 1.608 mm^3/wk. Similarly, BMC increase by 0.100g/wk and 1.608g/wk respectively in 2 periods. Unlike the others, BMD kept constant from wk8–17 at 0.239 g/cm^3, then increased exponentially at 17wk by 1.559 g/cm^3/wk ($P < 0.001$, $R^2 = 0.884$). The data was also grouped according to the developmental age period: period 1 (wk0–4), period 2 (wk6–16), and period 3 (wk17–21). The differences among periods were tested using the GLM procedure. The correlation among the parameters were evaluated by using CORR procedure. The analysis showed that, all parameters were different ($P < 0.05$) from each other among the periods, and the largest difference was between periods 1 and 3. Also, BW had a strong correlation with TV, BV, and BMC up to wk 17 ($P < 0.001$; $R^2 > 0.975$), and a moderate correlation after wk17 ($P < 0.05$, $R^2 = 0.449$ to 0.607). However, BW has moderate correlation with BMD up wk4 ($P < 0.001$, $R^2 = 0.603$), but no correlation ($P > 0.05$) with BMD during wk 6–16. In conclusion, there were dramatic changes in bone developing pattern at the beginning of laying (18wk), evidenced by the significant increases in BV, BMD and BMC. This indicates that calcium and other minerals are mobilized in a significant manner from bones before laying for egg shell formation, and layer nutrition at the point of producing the first egg may be critical for egg production and skeletal integrity during the later laying period.

Key Words: Modeling, layers, bone development pattern, linear, exponential

111 Prediction of feed intake in broilers using physical-chemical properties of diluted feeds. M. Nascimento*[1], N. Sakomura[1], L. Soares[1], G. Viana[1], M. Reis[1], and R. Gous[2], *[1]UNESP, Jaboticabal, Brazil, [2]University of Kwazulu-Natal, Pietermaritzburg, South Africa.*

One of the factors that can prevent an animal to consume a sufficient amount of a given food to satisfy your requirement is the physical capacity of the gastrointestinal tract. This can constrain bird food consumption especially when foods have low-density characteristics, the low concentration of nutrients or when in environments where the temperature is lower than their thermal comfort range, the need for energy exceeds its demand. Thus, this research had the objective of correlating the physical and chemical characteristics of a diet with the capacity of the gastrointestinal tract of broilers, through the daily food intake of the diet. Three trials were conducted, one for each phase (Initial: 1 to 14 d, Grower: 15 to 28 d and Finisher: 29 to 42 d). For each trial, 225 birds of Cobb500 strain were used, distributed in a completely randomized design, with 5 diluents and 5 dilution levels, totaling 25 treatments and 9 replications containing one bird each. A balanced diet (BD) was formulated to meet the requirement of all nutrients. The bulk diets were formulated diluting BD with 2.5%; 5%; 10% and 15% of cellulose fiber, sawdust, rice hulls, sand, and vermiculite. Diets were analyzed as one-way ANOVA. The models to predict the FI were elaborated from the determination of the correlation coefficients between the laboratory analyzes performed and the FI of the animal. For this, multiple regression analyzes were performed between the FI (FI, g/ $BWkg^{0.67}$/day) and laboratory analyzes to fit the model and determine the coefficients. Therefore, the variables that presented the highest correlation with FI were the inverse of the water holding capacity (1/WHC) and crude fiber (CF). Thus, for each phase (initial, growth and finishing) the intercepts (I) and coefficients for these variables were generated as a function of FI. For the initial, grower, and finisher phases the equations fitted to predict FI were: FI = 4.6 – 17.6 x (1/WHC) – 0.14 x (CF); FI = 101.62 – 131.11 x (1/WHC) – 0.91 x (CF), and FI = 151.86 – 196.45 x (1/WHC) – 2.5 x (CF), respectively.

Key Words: bulk capacity, water-holding capacity, crude fiber

112 Determining the effects of feeding two broiler strains varied crumble size and intact pellets (d 0-18) on starter and overall (d 0-62) performance. M. Lemons*[1], C. McDaniel[1], J. Moritz[2], and K. Wamsley[1], *[1]Mississippi State University, Mississippi State, MS, [2]West Virginia University, Morgantown, WV.*

Our laboratory found that feeding an average crumble (C) particle size of 2200 to 3736 µm improved starter performance; the greatest benefit was associated with the largest particle size tested (3736 µm). In this study, we investigated feed quality (FQ), in terms of the range of feed particles, due to the belief that chicks were self-adapting to larger particle sizes of feed. Additionally, due to starter performance being maximized at a particle size near a pellet (4,000 µm), it was of interest in this study to feed pellets to starting chicks, in addition to C; thus also focusing on feed form (FF). Lastly, to relate this research to a broader audience, we decided to utilize 2 strains (S). Therefore, the objective of the current study was to evaluate the effects of varying C particle sizes and intact pellet (IP) percentages presented during the starter growth phase (d 0–18) on starter performance using 2 S. The potential of a carryover effect on overall performance due to FF and FQ fed in the starter phase was also examined. The current study employed a 2 S × 2 FF × 3 FQ factorial arrangement within a RCBD. The 2 FF, C or IP, were fed as one of 3 FQ: Low-2210 µm (C) or 40% IP, Medium-3010 µm (C) or 60% IP, and High-3388 µm (C) or 80% IP. A common diet was made at a commercial mill, with modifications performed to adjust desired FQ for each FF. Common diets, also from a commercial mill, were fed after 18 d. Straight-run chicks (1,080/S) were randomly allocated by S to 1 of 108 pens. While the main effect S was significant, data in this abstract will focus on the FF × FQ interactions. Day 0–18 BW and BW gain demonstrated similar performance among C treatments, whereas BW and BW gain was improved as IP increased; birds fed 40% IP resulted in the lowest BW and BW gain ($P < 0.05$). A trend in d 0–18 FCR exhibited a similar response with reductions in FCR as IP increased ($P = 0.07$). For 0–32 d, FCR was improved if 3388 µm C were fed to birds, whereas birds fed all IP and C of 2210 or 3010 µm performed similar ($P = 0.03$). For 0–62 d, no significant differences were found for FF × FQ, FF or FQ ($P > 0.05$). These data suggest that FQ and FF presented in the starter growth phase are important when considering the length of the growout. Specifically, integrators in the "fast-food" market should feed 3388 µm C (not IP) from 0 to 18 d due to apparent d 32 FCR benefits. Integrators in the "cut-up" market may be less concerned with FF and FQ from 0 to 18 d, allowing C or IP to be fed; due to the absence of carry over effects at d 62. Additionally, while not discussed in the abstract, main effects and interactions were seen for S, making recommendations for FF and FQ complex depending on length of the growout and S used.

Key Words: pellet, crumble, particle size, feed form, feed quality

113 Implementation of net energy evaluating system in layer hens: Validation by performance and egg quality. S. B. Nafari*, *University of New England, Armidale, NSW, Australia.*

Apparent metabolisable energy corrected to zero nitrogen retention (AMEn) is widely used to formulate poultry feed. This system does not consider heat increment (HI) which is the dissipated heat from the body during feed digestion. The net energy (NE) system considers the HI of diets and gives an opportunity to formulate the poultry diets more accurately. Analysis with respiratory chamber was carried out to measure the heat production and energy partitioning of a wide range of diets to produce a net energy regression equation based on chemical composition of feed. A validation field study was then carried out using 600 Hy-Line Brown laying hens of 22 weeks of age for 20 weeks in a completely randomized design to examine whether diet formulated with NE is more efficient than that formulated with AMEn. Wheat, SBM, canola meal, wheat millrun, canola oil based diets were formulated with different ratios of the same ingredients and fed to 200 birds per treatment with 10 replicates each. Performance and egg quality data were subjected to ANOVA and separated with Duncan's multiple range test. The AMEn (MJ/kg), NE (MJ/kg), NE:AMEn, crude protein (CP) (g/kg), ether extract (EE) (g/kg) and added canola oil (g/kg) of the 3 experimental treatments were, respectively: T1)10.80, 8.25, 0.764, 169, 22, 3.3, T2)10.89, 8.32, 0.764, 166, 55, 19.2 and T3)11.39, 8.74, 0.768, 163, 66, 31.8. The high NE:AMEn (T3) birds showed higher values for egg weight (EWT; $P < 0.01$) and egg mass (EM; $P < 0.05$) and lower feed intake and feed conversion ratio (FCR; $P < 0.01$) compared with other groups. Birds fed the treatment with higher AMEn and NE (T2) than control (T1) had no differences in EWT, EM and FCR. Total egg number, hen day production (%) and body weight were not affected by treatment ($P > 0.05$). Birds fed the T3 diet had higher values for albumen % and yolk color but lower values for yolk % compared with T1 and T2

($P < 0.01$). Birds fed diet T2 had the lower Haugh units compared with other treatments ($P < 0.01$). Egg shell color score, egg shell breaking strength, shell deformation and egg shell thickness were unaffected by dietary treatments ($P > 0.05$). It was shown that formulation on an NE basis may result in diets that are more efficient.

Key Words: net energy, laying hen, performance, egg quality, validation

114 Dietary long-term storage corn resulted in a decrease of antioxidant status and egg quality of laying hens. L. Zhou*, X. Ding, J. Wang, S. Bai, Q. Zeng, Z. Su, and Y. Xuan, *Animal Nutrition, Wenjiang, Chengdu, Sichuan, China.*

Long-term storage corn (SC) changed the chemical composition of corn; however, effect of Long-term storage corn on laying hen is not clear. The objective of this study is to investigate the effect of SC on the antioxidant status and egg quality of laying hens. Four treatments were used under a 2 × 2 factorial arrangement with 2 types of corn (Regular and SC), and 2 levels of soy oil (0 and 2%) in diets. A total of 144 Lohmann commercial laying hens (51–wk-old) were fed the trial diets as corn-soybean meal-based diets for 8wk, then 18 eggs from each treatment were collected and stored under normal retail storage conditions (20 ± 4°C) for 21days, antioxidant status of laying hens and malondialdehyde (MDA) content of stored eggs were analyzed. All data were analyzed by 2-way ANOVA, and the main effect together with their interactive was determined in final model. No effect of soy oil was observed to alter the antioxidant status and egg quality ($P > 0.05$). Dietary SC with the addition of soy oil decreased the yolk index of fresh eggs compared with the dietary SC without the addition of soy oil. Dietary SC significant increased ($P < 0.05$) the malondialdehyde (MDA) level of egg yolk storage for 14d and markedly increased ($P < 0.01$) the MDA level of egg yolk storage for 21d. In conclusion, dietary SC decreased the antioxidant status and stored egg quality. Dietary SC decreased ($P < 0.05$) the liver superoxide dismutase and glutathione peroxidase (GSH-Px) activity, and ovary malondialdehyde (MDA) and GSH-Px activity.

Key Words: laying hen, long-term storage corn, antioxidant status, egg quality

115 Dietary peroxidized lipids alter immune cell recruitment in broilers. K. Fries*[1], M. Meyer[1], B. Kerr[2], and E. Bobeck[1], *[1]Iowa State University, Ames, IA, [2]USDA-ARS National Laboratory for Agriculture and the Environment, Ames, IA.*

Alternative energy sources such as restaurant oil are becoming more common in poultry diets to reduce the cost of production. Sustained heat results in peroxidation and structural changes that may elicit an immune response; however, the effects of feeding peroxidized lipids have not been well-documented in poultry. The objective of this work was to evaluate the effects of lipid source and peroxidation on ileal histomorphology and cytokine expression in the liver and intestine of broilers. Two hundred day-old Ross 308 broilers were housed in 40 battery cages (5 birds/cage) and randomly assigned to diets in a 4 × 2 factorial arrangement. Diets consisted of 4 oils (palm, soybean, flaxseed, and fish; 5% inclusion rate) ± heating (72 h at 90°C). Birds were euthanized on d 24 for tissue collection. Liver samples were analyzed for interleukin (IL) 1β, IL-6, IL-10, IL-18, interferon (IFN)- γ, and peroxisome proliferator-activated receptor (PPAR)- γ expression by quantitative PCR. Ileal samples were fixed, embedded in paraffin, and H&E stained for measurement of villus height, crypt depth, and analyzed by RNAscope to identify T cell presence and cytokine production. Expression of IL-1β, IL-4, IL-6, IL-10, IFNγ, CD3, and CD4 in the ileum was quantified by HALO software. Data were analyzed using SAS MIXED procedure with lipid source, peroxidation, and the interaction as fixed effects with significance at $P \leq 0.05$. Oil peroxidation resulted in a trend of decreasing villus height and crypt depth ($P = 0.06$ and 0.10, respectively). Lipid source, peroxidation, or lipid × peroxidation did not alter expression of IL-6, IL-10, IL-18, IFNγ, and PPARγ in liver (PCR; $P > 0.05$), while IL-1β resulted in trends (lipid, $P = 0.08$; interaction $P = 0.07$). Peroxidation significantly decreased the percentage of ileal cells expressing both IFNγ and CD3 by 6.08% ($P = 0.02$) while increasing the percentage of cells expressing both IL-6 and CD3 by 1.56% ($P = 0.02$). All lipid sources significantly affected the expression of IFNγ, with fish and palm oil producing less IFNγ than flaxseed and soy ($P = 0.002$). Peroxidation and lipid × peroxidation of flaxseed oil significantly increased expression of IL-6 by 3.60 and 11.16% ($P = 0.02$ and < 0.0001, respectively). Peroxidation decreased percentages of CD3+ cells in an interaction with cytokine production by 10.62% (IL-6; $P = 0.006$), 7.56% (IL-10; $P = 0.04$), 12.87% (IL-1β; $P = 0.001$), 25.87% (IFN-γ; $P = 0.004$). There were no significant changes in the expression of CD4, IL-4, IL-10, and IL-1β in the ileum. Overall, the results indicate peroxidation tended to change ileal histomorphology, and RNAscope may be a more useful method than PCR for the detection and evaluation of immunological changes in response to diet.

Key Words: lipids, peroxidation, immunology, broiler, RNAscope

116 Influence of soybean oil thermally-processed in the absence or presence of a liquid antioxidant when fed to broilers with or without an in-feed antioxidant. A. Gautier*[1], C. Ruan[1], B. Kerr[2], K. Vignale-Pollock[3], B. Kremer[3], C. Owens-Hanning[1], and S. Rochell[1], *[1]University of Arkansas, Fayetteville, AR, [2]USDA National Laboratory for Agriculture and the Environment, Ames, IA, [3]Kemin Industries Inc., Fayetteville, AR.*

Supplemental oil sources used in all vegetable-based poultry diets typically contain a high proportion of unsaturated fatty acids that are susceptible to lipid peroxidation. An experiment was conducted to evaluate the effect of feeding soybean oils that were thermally processed in the absence or presence of a liquid antioxidant (RENDOX CQ, Kemin Industries, Inc.) in combination with an in-feed antioxidant (ENDOX, Kemin Industries, Inc.) on the growth performance and carcass characteristics of male Ross × Ross 708 broilers. Six dietary treatments consisted of a factorial arrangement of 3 oil types (unprocessed, thermally processed, or pretreated with RENDOX CQ and thermally processed) and 2 in-feed antioxidant supplementation levels (0 or 0.01% ENDOX). Soybean oil was thermally processed at 65°C for 5.5 d and sampled immediately before feed mixing for analysis of lipid peroxidation markers. Soybean oils used in the starter phase that were unprocessed, thermally processed, or pretreated with RENDOX CQ and thermally processed had peroxide values of 2.4, 61.2, and 2.4 meg/kg, hexanal values of <5, 46, and <5 ppm, and 2, 4-decadienal values of <5, 514, and 8 ppm, respectively. The same general trend was observed among oils used in subsequent feeding phases and indicated that lipid peroxidation of oil was induced by thermal processing and prevented by pretreatment with RENDOX CQ. However, only minimal increases in lipid peroxidation markers were observed in the complete feeds. All experimental diets were formulated to contain 3% oil and were fed in 4 phases that consisted of starter (0 to 14 d), grower (15 to 28 d), finisher 1 (29 to 42 d), and finisher 2 (43 to 61 d) diets. Each experimental treatment was replicated with 12 pens of 18 broilers (1,296 total). Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were determined at the end of each feeding phase. At 62 d, 6 birds from

each pen were processed for evaluation of carcass characteristics and *Pectoralis major* fillets were scored for the incidence of woody breast (WB) and white striping (WS). Data were analyzed by 2-way ANOVA (SAS 9.4). No effects ($P > 0.05$) of oil type, ENDOX supplementation, or oil type × ENDOX supplementation interactions were observed for BWG, FI, or FCR of broilers for the overall experimental period of 0 to 61 d post-hatch. Likewise, dietary treatment did not influence ($P > 0.05$) processing measurements or severity of *P. major* myopathies. In conclusion, lipid peroxidation elicited by thermal processing was prevented by pretreatment of oil with RENDOX CQ, but oxidized soybean oil included at 3% of the diet did not impair the growth performance or meat quality of broilers.

Key Words: broiler, lipid oxidation, growth performance, meat quality, antioxidant

117 The Plackett-Burman design: An effective vehicle to evaluate multiple variables in poultry nutrition. A. Moss*[1], P. Chrystal[2], Y. Dersjant-Li[3], S. Liu[1], and P. Selle[1], *[1]The University of Sydney, Camden, NSW, Australia, [2]Baiada Poultry Pty Limited, Pendle Hill, NSW, Australia, [3]Danisco Animal Nutrition, DuPont Industrial Biosciences, Marlborough, United Kingdom.*

Plackett-Burman (PB) designs are effective as they permit the assessment of 11 factors in a single experiment. The PB design ranks factors by their impact on response parameters, which is valuable for identifying factors to be optimized in subsequent studies. This design is novel to animal studies; therefore, the objective of this study was to evaluate the PB design in the context of phytase supplemented broiler diets. Eleven dietary factors were screened over 12 treatments at 2 levels (canola meal [0 or 125g/kg], wheat or maize [600 g/kg], whole barley [0 or 200 g/kg], digestible lysine [9.6 or 11.4 g/kg], phytate-P [2.6 or 3.3 g/kg], Ca [6 or 10 g/kg], available P [3.0 or 4.5 g/kg], Na [1.6 or 2.0 g/kg], xylanase level 1 [0 or 1200 U/kg], xylanase level 2 [0 or 2000 U/kg], xylanase level 3 [0 or 2000 U/kg]; 3 xylanase factors were used to create a titration of xylanase activities derived from *Trichoderma reesei* of 0 to 5200 U/kg). Steam-pelleted diets based on wheat/maize and soybean meal with 1000 FTU/kg *Buttiauxella* phytase were offered to 468 male Ross 308 chicks (6 birds/cage, 6 replicates/treatment) from 7 to 28 d post-hatch. The effects of dietary treatments on weight gain, feed intake, gain:feed, relative gizzard weights, contents and pH were determined. Data were analyzed in JMP Pro 13 (SAS Institute Inc. JMP Software, Cary, NC) via PB screening methodology, with significant differences when $P \leq 0.05$. Digestible lysine level was the most important positive factor influencing weight gain, feed intake and gain:feed ($P < 0.001$). The high digestible lysine level increased gain by 10.2% (1228 versus 1419), intake by 4.92% (1889 versus 1982) and gain:feed by 10.3% (0.649 versus 0.716) compared with the low level. Ca was the most important negative factor influencing growth performance ($P < 0.001$). The high Ca level depressed gain by 6.36% (1367 versus 1280), intake by 3.85% (1974 versus 1898) and gain:feed by 2.60% (0.692 versus 0.0.674) compared with the low level. Whole barley was the most important factor positively influencing gizzard weights ($P < 0.001$) and gizzard contents ($P < 0.006$); the 200 g/kg inclusion increased gizzard weights by 24.8% (19.43 versus 24.25) and contents by 16.0% (7.68 versus 8.91) compared with ground grain diets. Ca was the most important factor influencing gizzard pH ($P < 0.002$) where the high Ca level increased pH (2.78 versus 3.01) compared with the low level. In conclusion, the PB design is a novel, effective vehicle to assess multiple factors in feeding studies for poultry.

Key Words: Plackett-Burman, screening design, broiler, nutrition

118 Supplemental dietary DHA-rich microalgae affected growth performance, health status and meat quality of broiler chicks. T. Sun*, S. Tolba, A. Magnuson, G. Liu, and X. G. Lei, *Cornell University, Ithaca, NY.*

Docosahexaenoic acid (DHA) is an n-3 polyunsaturated fatty acid. This study was to investigate effects of feeding a DHA-rich microalgal biomass on growth performance, health status, and meat characteristics in broilers. A total of 190 (day-old) Cornish male chicks were housed in an environmental control room (6 cages/treatment, 8 chicks/cage), and fed a corn-soybean meal basal diet supplemented with the microalgal biomass (*Aurantiochytrium*, Heliae, Gilbert, AZ) at 0, 1, 2, and 4% (0, 1.7, 3.4 and 6.8 g DHA/kg diet) for 6 weeks. Growth performance was measured weekly. Blood samples were collected at wk 3 and 6 (2 chicks/cage). Liver, breast, thigh and adipose tissue were sampled (2 chicks/cage) for biochemical and meat quality analysis. Data were analyzed by one-way ANOVA and linear regression analysis. Growth performance was not affected by 1 or 2% microalgae compared with the control, but 4% microalgae decreased ($P < 0.05$) body weight gain (19%) and feed conversion ratio (19%) during wk 4–6. The microalgae treatments resulted in dose-dependent decreases ($P < 0.05$, $R^2 = 0.21$- 0.54) in plasma alanine amino transferase activity and glucose, cholesterol and nonesterified fatty acid concentrations, but had little effect on plasma activity of alkaline phosphatase or concentrations of inorganic phosphorus, uric acid and triglyceride at wk 3 and 6. The carcass dressing percentage was not affected by the microalgae supplementation, but breast muscle weight was 21% lower ($P < 0.05$) in chicks fed 4% microalgae than the control. The microalgae supplementation caused linear increases in lipid peroxidation ($P < 0.01$, $R^2 = 0.62$–0.90) and hardness and chewiness ($P < 0.01$, $R^2 = 0.34$–0.44) of breast and thigh muscles, although springiness, pH, water holding capacity, and lipid profiles of both muscles in the microalgae-fed chicks remained similar to the control. Nonesterified fatty acid concentrations in liver and adipose tissue showed linear decreases ($P < 0.05$, $R^2 = 0.24$–0.28) in response to the microalgae inclusions. In conclusion, feeding chicks with 3.4 g DHA/kg diet (up to 2% microalgae) had positive or no adverse effects on growth performance, health status, and meat characteristics Study supported in part by Heliae, Gilbert, AZ, DOE (DE-EE007091), and Cornell University (Hatch grant NYC-127419).

Key Words: broiler, DHA, growth performance, meat quality, microalgae

119 Egg production, egg quality and visceral organ weight in Shaver White layers fed defatted black soldier fly larvae meal as total replacement of soybean meal from week 28 to 43 of age. Z. Mwaniki* and E. Kiarie, *University of Guelph, Guelph, ON, Canada.*

Insect meal has been proposed as an alternative and sustainable feedstuff. We investigated the effects of replacing soybean meal (SBM) with defatted black soldier fly larvae meal (BSFLM) in a corn-based diet fed from 28 to 43 wks of age. The sample of BSFLM (Enterra Feed Corp., BC, Canada) had concentration of 59.3, 7.0 and 6.1% DM for CP, fat and starch, respectively. A control corn-soybean meal diet was formulated to meet specifications, 2 additional diets were made by inclusion of either 10 or 15% BSFLM. Inclusion of SBM was 18.8, 5.2 and 0.0% in the control, 10% BSFLM and 15% BSFLM diets, respectively. Diets were iso-nutritious and the target AME, CP, SID Lys, SID Met and Ca was 2,750 kcal/kg, 15%, 0.75%, 0.45% and 4.3%, respectively and were prepared in pellet form. A total of 108, 28-wk old Shavers White hens were placed in conventional cages (6 birds/cage) and allocated diets to give 6 replicates/diet. The birds were allowed free access to feed and water to wk 43. Egg production on cage basis was

monitored daily. Feed intake and body weight was monitored in 4-wk intervals. All eggs laid on 6th d of wk 31, 35, 39 and 43 were used to measure Haugh units (HU), yolk color (YC), shell breaking strength (SBF), shell thickness (ST). Two birds per cage were sacrificed at the end of the experiment for liver, pancreas, gizzard, small intestine and ceca weights. The data was subjected to one way ANOVA and contrast coefficients for BSFLM response.There was no ($P > 0.05$) diet effect on hen d egg production, feed intake and HU. Egg weight and mass decreased quadratically ($P < 0.03$) with addition of BSFLM; the egg mass for the control, 10% BSFLM and 15% BSFLM was 55.9, 53.9 and 54.0 g/d, respectively. Feeding BSLFM linearly increased ($P = 0.045$) FCR (feed intake/egg mass), the observed FCR was 1.91, 1.99 and 2.02 for the control, 10% BSFLM and 15% BSFLM, respectively. There was no ($P > 0.05$) interaction between diet and sampling time point on egg quality parameters. Feeding BSFLM linearly ($P < 0.01$) increased yolk color intensity. A linear ($P < 0.01$) and quadratic ($P = 0.03$) increase in SBF and ST was observed with increasing BSFLM levels. Inclusion of BSFLM quadratically ($P \leq 0.01$) reduced empty ceca weight and increased liver weight and had no effect ($P > 0.05$) on pancreas, gizzard and small intestine weight. In conclusion, complete replacement of soybean meal with defatted BSFLM resulted in poor FCR linked to lower egg weight suggesting some amino acids may have been limiting. Improved yolk color suggested BSFLM had pigments that increased intensity of yolk color and better shell quality indicated potential role in calcium metabolism. Effects of BSFLM on liver and ceca weights warrant further investigations.

Key Words: black soldier fly larvae meal, egg production and quality, FCR, organ weight

120 Effects of supplemental docosahexaenoic acid and choline in hen diet on egg production and lipid metabolism. J. Yonke* and G. Cherian, *Oregon State University, Corvallis, OR.*

The objectives of the current study were to 1) investigate the effect of choline and a high-docosahexaenoic acid (C22:6 n-3, DHA) microalgae product (MAL) in the hen diet on production performance and egg physical and nutritional qualities, and 2) evaluate changes in hepatic lipid metabolism and oxidative status. Fifty-six, 26-week-old, White Leghorn hens were kept in individual cages and randomly allocated to one of the 4 dietary treatments, each with 7 replicate of 2 hens (n = 7 per treatment). The experimental diets were corn and soybean meal based, with 0% MAL (Control), 1% MAL and no additional choline chloride (MA), and diets Ch0.1 and Ch0.2 contained 1% MA and 0.1 and 0.2% supplemental choline chloride, respectively. All diets were isocaloric, isonitrogenous and contained the same amount of vitamin and mineral premix, which provided 551 mg/kg choline in the diets. The feeding trial lasted 16 weeks after a 2 week adaptation period. Eggs were collected, counted and weighed daily, feed intake and egg quality were measured every 2 weeks, and fatty acid (FA) composition was analyzed every 4 weeks. Birds were euthanized for tissue collection at the end of the trial. The effects of MAL and choline chloride on all response variables was analyzed as a mixed linear model with repeated measures. Using orthogonal contrasts, MA was compared with Control to calculate the effect of MAL, and Ch0.1 and Ch0.2 were compared separately to MA to calculate the affect of choline chloride dosages in diets containing MAL. Compared with Control, MA increased egg yolk content of DHA ($P < 0.0001$), phosphatidylethanolamine (PE) ($P = 0.02$), and phosphatidylcholine (PC) ($P < 0.0001$). In the liver, MA similarly increased PE ($P = 0.0003$) and PC ($P = 0.0003$) concentrations, but also increased lipid peroxidation products ($P = 0.01$). Compared with MA, Ch0.1 increased hen day egg production ($P = 0.03$), daily egg mass ($P = 0.02$), Haugh unit ($P = 0.04$), and total lipid ($P = 0.04$) and γ-tocopherol (γT) ($P = 0.05$) concentrations in egg yolks. Additionally, Ch0.1 increased hepatic concentrations of α-tocopherol (αT) ($P = 0.03$) and γT ($P = 0.005$), and decreased feed conversion ratio ($P = 0.005$) and hepatic lipid peroxidation products ($P = 0.005$). Ch0.2 likewise increased hepatic γT ($P = 0.0002$) and αT ($P = 0.001$) concentrations, but did not produce any of the other changes associated with Ch0.1. DHA content of eggs can be greatly increased by feeding MAL. Supplementing 0.1% choline chloride in hen diets containing MAL can improve production performance and egg quality and protect the liver from oxidative stress. Adding 0.2% choline chloride, however, may reverse some improvements.

Key Words: choline, n-3 fatty acid, laying hen, algae, phospholipid

121 Effect of mercury chloride on laying performance, egg quality, renal histopathology, and oxidative stress in laying hens. Y. Ma* and X. Zou, *Zhejiang University, Hangzhou, China.*

The objective of this study was to determine the effect of mercury chloride on laying performance, egg quality, renal histopathology and oxidative stress in laying hens. Antioxidant enzyme genes and the nuclear factor erythroid 2-related factor 2 (Nrf2)-Kelch-like ECH associated protein 1 (Keap1) signal pathway were further studied to uncover the molecular mechanism. A total of 512 40-week-old Hy-Line Brown laying hens were randomly allocated to 4 groups with 8 pens per group and 16 hens of each pen. The birds were fed with 4 diets containing graded levels of mercury (Hg) at 0.270, 3.315, 9.405, and 27.230 mg/kg, respectively. Results revealed that both laying performance and egg quality were significantly decreased ($P < 0.05$) after Hg exposure. With increasing dietary dosage of Hg, accumulation of Hg in viscera was significantly increased ($P < 0.05$), and histopathological damages in kidney were more and more severe. Blood urea nitrogen, uric acid and creatinine were significantly increased ($P < 0.05$) in all Hg-treatment groups. Besides, the activities of superoxidative dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GSH-Px), and glutathione (GSH) content all significantly decreased ($P < 0.05$), while malondialdehyde (MDA) content significantly increased ($P < 0.05$) in kidney after Hg exposure. In addition, positive relationships occurred between antioxidant enzyme activities and antioxidant gene expressions or between antioxidant gene expressions and Nrf2 mRNA expression, while negative correlations occurred between Nrf2 and Keap1 at transcription and protein levels. It could be concluded that dietary Hg could reduce laying performance and egg quality, and induced renal function disorders and oxidative stress by means of impairing Nrf2-Keap1 signal pathway in laying hens.

Key Words: kidney, laying hen, mercury, Nrf2-Keap1 signal pathway, oxidative stress

122 Effect of biochar and *Miscanthus* spp. as litter treatments and feed form in male turkeys. K. Flores*, J. Grimes, and A. Fahrenholz, *North Carolina State University, Raleigh, NC.*

Litter is important for birds' performance, health, and environmental impact. Pine shavings are the most common bedding for poultry. However, the increase in its price and its scarcity have created new research opportunities for reusing litter bedding. Increased fines in the feed have been found to decrease performance to 3 weeks; however, data is needed to determine the effect of fed fines to market age. The objective of this study was to determine how biochar and Miscanthus grass in the litter and reducing the amount of fines in the feed affect performance, small

intestine morphology, ammonia production and litter microbiology. Eight hundred twenty-seven Nicolas Select male poults were randomly assigned to 48 concrete-floor pens. The experimental design was a completely randomized block design with a 2 × 4 factorial arrangement of 2 levels of fines in the feed (screened vs non-screened) and 4 litter mixes using old brooder litter (70% in all) and a combination of biochar and Miscanthus grass (0% biochar and 30% *Miscanthus* spp.; 5% biochar and 25% *Miscanthus* spp.; 10% biochar and 20% *Miscanthus* spp.; 20% biochar and 10% *Miscanthus* spp.). Bird body weight (BW), weekly body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), feed particle size, pellet durability index (PDI), ammonia production of litter, coliform count units in litter (cfu), and duodenum, jejunum, ileum and ceca morphology were determined, and differences were considered to be statistically significant at $P \leq 0.05$. Poults fed screened feeds were significantly different in FI (45.58 ± 0.08 kg), and FCR (2.205 ± 0.014 feed: gain) from 0 to 20wk of age. Litter treatment with 20% biochar resulted in higher BW from 11 to 20 wk of age (20.91 ± 0.16 kg). At 11 wk, less activity was needed to decake 20% biochar pens, which may correspond with a reduced stress level experienced by the birds. Moreover, standard method agar coliform cfu decreased significantly in the 20% biochar litter treatment at 20wk (7.85 ± 0.07 log cfu/g). No difference due to litter or screening levels were observed on litter ammonia emissions. In conclusion, lowering the amount of fines in the feed increased BW by increasing FI and improved FCR at 20wk. The inclusion of biochar at 20% and *Miscanthus spp*. at 10% did not affect litter ammonia emissions, but increased BWG at 11 wk (3.70 ± 0.05 kg).

Key Words: turkey, litter, *Miscanthus*, feed quality, biochar

123 Impacts of L-valine inclusion in two diets varying in ingredient profile on broiler growth performance and breast meat yield. A. Jasek*[1], K. Haydon[2], T. Lester[1], R. Latham[3], J. Lee[1], and R. Brister[3], *[1]Texas A&M University, College Station, TX, [2]CJ America, Downers Grove, IL, [3]Tyson Foods Inc., Springdale, AR.*

The objective of the current study was to evaluate the impact of the use of L-valine on growth performance and yield when included in 2 diets varying in ingredient profile. A total of 1,120 broilers were assigned randomly to 4 dietary treatments consisting of 10 replicates of 28 male Cobb 700 broilers per pen. The experimental design was a 2 × 2 factorial, containing 2 diet types, all vegetable (VEG) and a diet containing meat and bone meal (MBM). Diets were formulated in an effort to replicate diets being used currently and were not constrained to be equal in nutrient content. Presence of L-valine in the formulation allowed for a reduction in soybean meal in each diet of approximately 1% of the total diet. Broilers were fed starter (d1–14), grower (d14–28), and finisher (d28–48) diets. Average body weight (BW), body weight gain (BWG), mortality adjusted feed conversion ratio (FCR), feed consumption (FC) and mortality (%) were determined on d 14, 28, 42, and 48. On d 49, following an 8 h feed withdrawal, 8 birds per replicate pen were randomly selected for evaluation of processing yields. Data were analyzed via a 2 × 2 factorial design with main effect means deemed significant at $P \leq 0.05$. In the presence of a significant interaction, a one way ANOVA was conducted and individual treatment means separated using Duncan's Multiple Range Test. On d 14, the presence of L-valine reduced (6 g difference; $P = 0.018$) BW compared with broilers fed diets not containing L-valine , however on d 42 and 48, no difference in BW was observed between diets with and without L-valine . The VEG diet increased ($P < 0.05$) BW throughout the duration of the experiment compared with diets containing MBM which was attributed to increases ($P < 0.05$) in FC. No differences were observed in cumulative FCR (d 1–42 and 1–49) between diet type or L-valine inclusion. No differences were observed in carcass and breast weight or yield between diet type or L-valine presence. Additionally, woody breast and white striping were not impacted by diet type or L-valine presence. These data confirm that including L-valine in dietary formulation to reduce soybean meal inclusion has no negative impact on performance or breast meat yield and can potentially lower diet cost.

Key Words: valine, broiler, performance, breast yield

124 Nutritional and metabolic effects of isoleucine supplementation in low crude protein corn-soybean diets fed to Shaver white hens from 19 to 48 weeks of age. I. Parenteau*[1], M. Stevenson[2], A. K. Shoveller[1], and E. Kiarie[1], *[1]University of Guelph, Port Perry, ON, Canada, [2]Halchemix Canada Inc., Port Perry, ON, Canada.*

Isoleucine is speculated to be limiting in low CP (LCP) diets for layers, however, its specific requirements and metabolic dynamics are not well characterized. Therefore, the objective of the current study was to assess metabolic responses to Ile supplementation in a LCP diet fed to pullets from 19 to 48 weeks of age (woa). One hundred eighty Shaver White pullets were placed in cages (6 hens/cage) based on body weight (BW; CV < 4%) and allocated to either a standard CP control diet (HCP; 18% CP) or a LCP diet (16% CP) supplemented with graded levels of Ile (0.7, 0.8, 0.9 and 1.0 SID Ile:Lys). At 28 woa, the CP content was reduced by 2% to start phase 2. Diets were isocaloric and formulated on ideal protein concept with SID Lys of 0.75 and 0.71% in phase 1 and 2, respectively. Egg weight (EW) and feed intake (FI) were recorded biweekly whereas egg quality (Haugh unit and shell strength) and BW were recorded at 4-week intervals. Yolk to albumin ratio was evaluated in phase 2 only. At 29 and 48 woa, 2 birds/cage were randomly bled for plasma AA and serum uric acid (UA) measurements. Hens for plasma AA were subsequently sacrificed to record breast muscle and liver weights. Data were subjected to ANOVA in JMP 13.1.2, and specific contrasts for HCP vs. LCP and linear and quadratic responses to Ile were used in post hoc comparisons. The Ile intake of 636 to 831 mg/d/hen with increasing Ile:Lys was negatively associated ($P < 0.004$) with FI at higher Ile levels (≥0.9 SID Ile:Lys), resulting in a decrease of Lys intake from 933 to 896 mg/d/hen. At 29 woa, plasma AA paralleled FI with the lowest and highest concentrations seen in birds fed 0.9 and 0.8 SID Ile:Lys, respectively. There was a quadratic decrease ($P < 0.05$) in ketogenic AA, branched chain AA (BCAA) and UA in response to Ile, with the lowest values in hens fed 0.9 SID Ile:Lys. At 48 woa, there was no diet effect ($P > 0.05$) on UA, but plasma Ile increased linearly ($P = 0.01$) with additional Ile supplementation. Overall, eggs from birds fed 0.9 SID Ile:Lys were the heaviest ($P < 0.001$) and had the highest shell strength ($P = 0.004$), but were not different ($P > 0.05$) from the HCP control. Increasing Ile to 1.0 SID Ile:Lys resulted in higher BW and lower EW ($P < 0.01$) than birds fed HCP or LCP with lower supplemental Ile. Isoleucine linearly increased egg albumin ($P < 0.0001$) and Haugh unit ($P = 0.002$). No differences ($P > 0.05$) in breast and liver weights were detected between treatments. The data suggest that CP can be reduced by 2% without compromising performance or egg quality, and has the potential to reduce nitrogen emissions through improved amino acid utilization, if Ile is supplemented to a level of 0.9 SID Ile:Lys.

Key Words: layer, isoleucine, egg production, uric acid, amino acid metabolism

125 Effects of L-methionine supplementation on pullets and laying hens subjected to chronic cyclic heat stress. F. Castro*[1], H. Choi[2], and W. Kim[1], *[1]University of Georgia, Athens, GA, [2]CJ Corporation, Seoul, Korea.*

The objective was to evaluate different levels of total sulfur amino acids (TSAA) supplementation with L-Methionine (L-Met) on growth performance and bone development in pullets and laying hens subjected to chronic cyclic heat stress. For pullet phase (0–18wks), 216 Hy-Line W36 d old pullets were distributed in a completely randomized design, with 3 dietary treatments, 6 replicates of 12 birds. Birds were subject to 35°C/7h/d throughout this phase. For production phase (19–45wks), 144 Laying hens were equally-distributed by body weight within the same dietary treatment into 2 rooms (Heat stress room: HR vs. normal temperature room: CR). A completely randomized design arranged in a factorial design 2x3 was used, with 8 replicates of 6 birds. The main factors were diets and rooms. The dietary treatments used in both phases were: T1:70% TSAA without L-Met supplementation (basal diet), T2:85% TSAA+L-Met, and T3:100% TSAA+L-Met (Hy-Line W36 recommendation). Treatments were formulated to be isoproteic using glutamine to balance the Met addition. The rooms differed only in temperature: HR:32°C/8h/d and CR:21°C/24h/d. At wk 18 and 45 body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and number of eggs/hen/d (egg production) from 18 to 45wk were evaluated, and bone weight, volume, ash and density measured from one bird/pen. The means were analyzed by one and 2-way ANOVA and compared by

Tukey's test when significant ($P \leq 0.05$). From 0 to 18wk, FI was not significantly different between treatments ($P > 0.082$). Birds fed 70% TSAA showed the lowest BWG and worst FCR compared with the treatments with L-Met supplementation ($P < 0.001$). Bone volume, weight, ash (%) and bone density were not affected by the dietary treatments ($P > 0.207$), whereas ash content (g) was significantly different. The treatments with L-Met supplementation resulted in higher ash content than the treatment with 70% TSAA ($P = 0.045$). From 19 to 45wks, no interactions were found for BWG, FI, egg production, and bone traits ($P > 0.331$). BWG was the lowest for 70% TSAA ($P < 0.001$). FI was the lowest for 70% TSAA, and birds in CR ate the most ($P < 0.004$). Egg production was the lowest for 70% TSAA and HR ($P < 0.002$). Bone volume from birds fed 100% TSAA+L-Met had higher volume compared with 70% TSAA, and bones from HR had higher volume ($P < 0.041$). There was a significant interaction for FCR ($P = 0.045$). Birds fed 70% TSAA in HR had the highest FI, followed by 70% TSAA in CR, then the other dietary treatments, which were similar among themselves. In conclusion, Met supplementation is fundamental to maintain good performance and bone development, since Met is an essential amino acid and bone matrix proteins are important for bone development and mineralization.

Key Words: bone quality, growth performance, laying hen, heat stress, L-methionine

126 Effects of amino acid reduction on growth performance of 6 strains of broilers. B. Zhang*, X. Wang, and Wei Zhai, *Mississippi State University, Mississippi State, MS.*

The effects of amino acid (AA) levels on the growth performance of 6 strains of broilers were determined. A total of 1,504 broilers were randomly allocated to 8 blocks in an environment controlled house. Each block contained 12 treatment pens with 16 birds per pen. The treatments were arranged in a 6 (strains) × 2 (diets) factorial layout. The 6 strains included 5 modern commercial broiler strains (Hubbard × Cobb 500, CobbMx × Cobb 500, Cobb MV × Cobb 700, Ross 308, and Ross 708) and a control broiler strain developed in 1955, Athens Canadian Random Bred (ACRB). The birds were fed one of 2 diets: a diet with a normal level of AA (meet or exceed the nutrient requirement of all commercial broilers according to primary breeder recommendations) or a 20% AA reduced diet (20% lower digestible lysine, total sulfur AA, and threonine). Data were analyzed using 2-way ANOVA of the PROC GLM in SAS 9.4. Mortality was not affected by strain ($P = 0.391$) or dietary treatment ($P = 0.902$) from d 0 to 55. As compared with birds fed AA reduced diets, birds of the 5 commercial strains (except for ACRB) fed normal AA diets exhibited higher BW on d 14, 28, and 41 (with all $P < 0.0001$); and exhibited higher BW gain from d 0 to 14, 14 to 28, 0 to 28, and 0 to 41 (with all $P < 0.0001$). The lack of response of ACRB to AA reduction may due to their lower nutritional requirements; the AA reduced diets might still meet their AA requirements. Birds of each strain fed normal AA diets exhibited lower FCR from d 14 to 28 ($P < 0.0001$), 0 to 28 ($P < 0.0001$), 0 to 41 ($P = 0.037$), and 0 to 55 ($P = 0.036$) than birds of same strain fed AA reduced diets. Amino acid levels exhibited different effects on BW of the 6 strains on d 14, 28, 41, and 55 (with all $P < 0.0001$). Amino acid reduced diets decreased BW on d 55 of 2 strains ($P < 0.0001$), but not the other 4 strains, when compared with normal AA groups. Interestingly, BW gain from d 41 to 55 were increased in one of the commercial broiler strains but decreased in another strain with dietary AA reduction, with others unchanged ($P = 0.017$). These different responses on BWG may due to their different growing speed of certain strains and their related AA requirements. Even fed same diets, 2 commercial strains that had similar initial BW exhibited different BW later ($P < 0.0001$). In conclusion, decreasing dietary AA slows down the growth of all 5 modern commercial broilers at early age, however, some of the strains were not sensitive to the AA reduction at later age. One particular strain even exhibited higher growth rate when fed lower AA diets at later age. Therefore, the effects of AA reduction on growth performance may depend on different nutritional requirements of different strains.

Key Words: amino acid, broiler, growth performance, strain

127 The effect of nutrient density on performance and processing yield in Cobb 700 mixed-sex broilers. C. Johnson*[1], R. Latham[2], R. Brister[2], R. Shirley[3], and J. Lee[1], *[1]Texas A&M University, College Station, TX, [2]Tyson Foods Inc., Springdale, AR, [3]Adisseo, Alpharetta, AR.*

In a 4-phase, 49 d-old experiment, the growth performance and breast yield of Cobb 700 × MV male, mixed-sexed broilers was evaluated after altering the dietary AA density and AME content of the diet in the last 3 feeding phases (grower: d 13–26, finisher: d 26–36, and withdrawal: d 36–49). The experimental design consisted of a 3 × 2 factorial design with 3 levels of AA density (low, moderate, and high) and 2 levels of energy (high and low) that were allotted in a randomized, complete block design, with 10 replicate pens/trt and 36 birds/replicate. The moderate AA density for the 3 phases consisted of 1.07, 0.96, and 0.89% dLys, with the low and high AA densities containing either a 7.5% decrease or increase in dLys relative to the moderate. The low energy level for the 3 phases consisted of 3062, 3106, and 3117 kcal/kg AME, with the high energy level consisting of low +110 kcal/kg AME. Trts within a phase were formulated to maintain equivalent dEAA: dLys ratios across all diets within a given phase. All birds were fed a common starter from 0 to 12 d. Body weight (BW), feed intake (FI) and mortality adjusted feed conversion ratio (FCR) were evaluated on d 12, 26, 36 and 49. On d 50, 4 males and 4 females from each pen were processed to determine fat pad and skinless-boneless breast and tender yields. Data were analyzed via 3 × 2 factorial ANOVA with differences deemed significant at $P \leq 0.05$, and main effect means separated using Duncan's Multiple Range test. Feed intake was higher in broilers fed the low AA density diet when compared with those fed the high AA density diet during the grower phase ($P < 0.05$). Reducing energy level during this phase also increased FI ($P < 0.05$). Increasing AA density increased BW on d 37 and 49, as broilers consuming the high AA diet exhibited elevated BW compared with those fed the low AA density diet ($P < 0.05$). Energy level did not impact BW. Feed conversion ratio was the most sensitive performance metric to adjustments in AA density and energy level. Significant, incremental decreases in FCR were observed with each increase in AA density in the grower, finisher, d 1–26, d 1–37 and d 1–49 ($P < 0.05$). Similar to AA density, increasing the energy content of the feed decreased FCR for the grower, finisher, d 1–26, d 1–37 and d 1–49 ($P < 0.05$). Similar to FCR, skinless-boneless breast yield increased with each incremental rise in AA density ($P < 0.01$); conversely, fat pad yield increased as AA density of the diet decreased ($P < 0.01$). The performance and meat yield of mixed-sex Cobb 700 × MV broilers respond positively to a higher density of dAA and energy.

Key Words: Cobb 700, broiler, energy density, amino acid density, breast yield

128 Effects of pre-starter diets varying in amino acid density given to broilers that received coccidiosis vaccination at hatch. S. Cloft*[1], S. Rochell[2], K. Macklin[1], and W. Dozier, III[1], *[1]Auburn University, Auburn, AL, [2]University of Arkansas, Fayetteville, AR.*

Coccidiosis vaccination induces immunity by eliciting a mild enteric challenge that causes growth depression in broilers. This depression has been shown to be reduced by increasing the digestible (dig) amino acid (AA) density in the starter diet. A study was conducted to determine if increasing dig AA density for a shorter duration would support the bird through the challenge without compromising performance. One thousand and 8 hundred male Ross × Ross 708 broilers were allocated to 60 floor pens (0.84m^2/bird) based on vaccination status. Each pen was assigned to 1 of 6 treatments with 10 replicate pens per treatment. Four pre-starter diets varying in dig AA density [Low (1.15% dig Lys), Moderate (Mod) (1.25% dig Lys), High (1.35% dig Lys), and Positive Control (PC) (1.15% dig Lys + Diclazuril)] were placed for the first 9 d. Then, 3 starter diets varying in dig AA density [Low (1.15% dig Lys), High (1.25% dig Lys), and PC (1.15% dig Lys + Diclazuril)] were given until 19 d of age when birds were given common diets for the remainder of the trial. All diets were formulated to similar dig ratios of Thr (0.67), TSAA (0.78), Val (0.70), Ile (0.67), Arg (1.05) and Trp (0.17) to dig Lys. Vaccinated birds received a 1x dosage of Coccivac- B52 by spray cabinet and non-vaccinated birds were given the PC diets. At 19 d of age, intestinal lesion scoring was conducted on 4 birds per pen to confirm vaccination. At 40 d of age, 14 birds per pen were processed for measurement of carcass traits. One-way ANOVA and pre-planned orthogonal contrasts were used to analyze treatment effects. At 19, 27, and 40 d of age, broilers that received the High and Mod AA pre-starter and High AA starter diets had increased BWG compared with broilers fed the Low AA diets ($P \leq 0.006$). At 27 d of age, the broilers receiving the PC diet had higher BWG than those fed the Low AA diets ($P < 0.001$). Broilers that received the Low AA pre-starter diet had a lower cumulative BWG than those fed the High and Mod AA pre-starters ($P = 0.007$). Broilers receiving the Mod and High AA pre-starter diets had a greater proportion of scores of 1 in the upper intestine as compared with the PC diets ($P \leq 0.03$). Broilers that received the High and Mod AA pre-starter and High AA starter diets had the heaviest breast weights ($P \leq 0.008$). The vaccination lead to a 28 g increase in total breast weight between the PC diets and the broilers fed the Low AA diets ($P = 0.03$). Increasing the pre-starter dig AA density increased total breast weight by 25 g (Mod) and 35 g (High) ($P \leq 0.03$). Results from this study indicated that providing a pre-starter diet with increased dig AA density improves growth and meat yield of broilers vaccinated against coccidiosis.

Key Words: broiler, coccidiosis, vaccine, amino acid, starter

129 Dietary glycine+serine and sulfur amino acids effects in broilers fed low-protein diets. P. Aguihe*[1], A. E. Murakami[2], I. C. Ospina-Rojas[2], K. C. Nunes[2], and E. Iyayi[1], *[1]University of Ibadan, Ibadan, Nigeria, Ibadan, Nigeria, [2]Universidade Estadual de Maringa, Maringá, Brazil.*

This study was conducted to determine the optimum dietary glycine+serine (Gly+Ser) levels in low-crude protein with different concentrations of the standardized ileal digestible (SID) methionine + cysteine (Met+Cys) for broiler chickens from 1 to 21 d of age. A total of 1,275 1-d-old Cobb-Vantress male broiler chicks were distributed in a 5 × 3 completely randomized factorial arrangement of 15 treatments with 5 replicate pens of 17 birds each. Treatments consisted of 5 levels of dietary total Gly+Ser (1.72, 1.87, 2.02, 2.17 and 2.32%) and 3 levels of SID Met+Cys (0.77, 0.90 and 1.03%, corresponding to 85, 100 and 115% of the required Met+Cys respectively). All data were analyzed using the GLM procedure of SAS software and analyzed by 2-way ANOVA to determine the main effects of Gly+Ser and SID Met+Cys levels and their interaction. At 21 d, interactions were observed ($P < 0.05$) between dietary Gly+Ser and Met+Cys levels for the feed:gain ratio and relative breast weight. Increasing Gly+Ser levels improved linearly ($P < 0.05$) the feed:gain ratio and relative breast weight in birds fed 0.77 and 1.03% SID Met+Cys diets but in 0.90% SID Met+Cys diets, these variables showed a quadratic effect ($P < 0.05$), with an optimization levels of 2.16 and 2.06% Gly+Ser for feed:gain ratio and relative breast weight respectively. The main effect of increasing total Gly+Ser levels resulted in a decreasing linear effect ($P < 0.05$) on feed intake and quadratic effect ($P < 0.05$) on ADG, FCR, muscle creatine content and breast weight with optimization point achieved at 2.28, 2.29, 2.11 and 2.22% respectively. Diets containing 0.90% SID Met+Cys improved ($P < 0.05$) the performance, relative breast weight and muscle creatine content than 0.77 and 1.03% SID Met+Cys diets. Therefore, a minimum of 2.10% total Gly+Ser concentration is needed to optimize the performance, pectoral muscles creatine and relative breast weight of broilers at 1 to 21 d of age fed varying SID Met+Cys in low protein, AA supplemented corn-soybean diets. Thus, lower and higher levels of SID Met+Cys increased the dietary total Gly+Ser requirement for broiler chickens fed low protein diets during the starter phase.

Key Words: glycine+serine, sulfur amino acid, low protein, performance, muscle creatine

130 Broiler fed a lower protein diet supplemented with synthetic amino acid did not impair growth performances but improved intestinal morphology for 35-day after hatch. W. H. D. S. Macelline*[1], S. Wickramasuriya[1], H. M. Cho[1], E. Kim[1], T. K. Shin[1], J. S. Hong[1], H. Choi[2], and J. M. Heo[1], *[1]Chungnam National University, Daejeon, Daejeon, Korea, [2]CJ CheilJedang Corporation, Seoul, Korea.*

The present study investigated the effect of lower protein diet supplementation with limiting amino acids up to the estimated requirement levels on growth performance, intestinal morphology and blood metabolites of the broiler chickens. Two hundred fifty-six one-day-old male broiler chicks (Ross 308) were randomly assigned in to one of 3 dietary treatments to give 8 replicates with 8 birds per pen. Three dietary treatments were; 1) High protein diet (HP; diet with 23.5% and 20.5% crude protein in starter and finisher phases respectively), 2) Lower protein diet (LP; diet with 18% and 17% of crude protein in starter and finisher phases respectively), 3) Low protein diet supplemented with synthetic amino acids to fulfil the ideal amino acids pattern up to required level (LPA). Therefore, HP and LPA diets were formulated to fulfil the least standard ileal digestible amino acids (Met, Lys, Thr, Trp, Val, Ile, Arg, Gly, and Ser) requirement. Growth performance were measured weekly for 35-d. Two birds per pen (n = 16) with closet to mean body weight were euthanized for collect blood and ileum tissue samples on d 35. Data were subjected to analyze using one-way ANOVA of general linear model procedure in SPSS (version 24). In starter phase (0–14 d), birds fed HP diet increased ($P = 0.001$) growth performances compared with birds fed LPA and LP diets. Nevertheless, broilers fed LPA diet showed similar growth performance compared with those fed HP diet during grower phase (15–35 d) and the overall period (0–35 d). Similarly, improved ($P = 0.001$) feed efficiency was observed that birds fed LPA diet compared with birds fed LP diet in overall period. Further, birds fed HP diet resulted in higher ($P = 0.036$) globulin and ($P = 0.035$) total protein concentrations compared with those were in LPA diet on d 35. Interestingly, no difference ($P = 0.213$) was observed among dietary treatments for blood urea nitrogen on d 35. Moreover, broilers fed HP and LPA diets showed increased ($P = 0.007$) ileum villus height and ($P = 0.003$) crypt depth values compared with broilers fed LP diet. In

conclusion, broilers fed a lower protein diet supplemented with synthetic amino acids up to estimated requirement levels maintained growth performance and intestinal morphology similar with broilers fed HP diet.

Key Words: broiler, growth performance, gut health, low protein diet, synthetic amino acids

131 Feeding a low protein diet supplemented with synthetic amino acids maintains growth performance while lowering the incidence of foot pad dermatitis in broiler chickens. S. Wickramasuriya*[1], W. H. D. S. Macelline[1], H. M. Cho[1], E. Kim[1], J. S. Hong[1], H. Choi[2], and J. M. Heo[1], *[1]Chungnam National University, Daejeon, Daejeon, Korea, [2]CJ CheilJedang Corporation, Seoul, Korea.*

A study was conducted to investigate the effect of a lower protein diet with supplementation of the limiting essential amino acids up to the estimated requirement levels on growth performance, visceral organ weights and foot pad dermatitis in broiler chickens. A total of 224, 1-d-old Ross 308 broiler chicks were randomly allocated into 3 dietary treatments to give 7 replicates per treatment with 8 birds per pen. Three dietary treatments were; 1) Sufficient protein diet (SP; a diet with sufficient protein and amino acids), 2) Low protein (LP; a diet with 5% and 3% lower protein levels and subsequent deficient essential amino acids of nutrient requirements of broiler chickens for starter and finisher phase respectively), and 3) a low protein diet fortified with essential amino acids up to the estimated requirement levels (LP+AA). All diets were formulated to at least contain the ideal pattern of ileal digestible AA except LP. Crystalline amino acids (e.g., Lys, Met, and Thr) were added to LP+AA, with Trp, Arg, Val, Ile, Gly and Ser to achieve an ideal pattern of amino acids. Body weight, feed intake and feed conversion ratio were measured on weekly basis. One bird per pen was selected and sacrificed for visceral organ weight measurement on d 35. At the end of the experiment, foot pad dermatitis of the birds was scored using a 5-point scale (Butterworth, 2013). All data were subjected to a one-way ANOVA using the GLM Procedure of SPSS and seemed significantly different at $P \leq 0.05$. Birds fed with LP+AA diet maintained growth performance ($P > 0.05$) compared with those fed a SP diet in grower phase (14–35 d) and the overall period (0–35 d) while it was higher ($P < 0.05$) than the LP diet fed birds in the same periods. Lower feed intake ($P < 0.05$) was observed with bids fed LP+AA and LP diets with compared with birds fed SP diet in grower phase and the overall period. Moreover, birds fed LP+AA diet showed higher ($P < 0.05$) feed efficiency over the birds fed LP diet for the entire experiment period and it was similar ($P > 0.05$) with birds fed SP diet. Nevertheless, no difference ($P > 0.05$) was observed among dietary treatments for visceral organ weight for the entire period of study. Birds fed with LP+AA diet showed a tendency ($P < 0.1$) for the lowest incidence of foot pad dermatitis compared with those fed the other dietary treatments (i.e., SP and LP). Therefore, our study indicated that feeding a low protein diet supplemented with crystalline amino acids up to the estimated requirement levels did not impair growth performance for hatch to 35-d of age along with the lower incidence of foot pad dermatitis of the broilers compared with those were in either SP or LP.

Key Words: broiler, foot pad dermatitis, growth performance, low protein diet, amino acids

132 Corn-expressed carbohydrase can improve performance and reduce digesta viscosity of broilers fed a high non-starch polysaccharide Diet. V. Ayres*[1], H. Baldwin[1], X. Li[3], H. Xu[3], M. Raab[3], J. Boney[2], J. Broomhead[3], and J. Moritz[1], *[1]West Virginia University, Morgantown, WV, [2]Pennsylvania State University, State College, PA, [3]Agrivida Inc., Woburn, MA.*

The inclusion of high non-starch polysaccharide (NSP) feed stuffs in broiler diets limit optimal broiler performance. Dietary additions of NSP degrading enzymes may improve digesta viscosity, nutrient digestibility, and thus broiler performance. Objectives of the current study were to determine the effects of a corn-expressed recombinant carbohydrase (AC1) on broiler performance and digesta viscosity in diets containing high NSP ingredients through 21 d of age. A total of 720 Hubbard × Cobb 500 straight-run, day-old chicks were assigned to one of 6 dietary treatments. Each treatment consisted of 12 replicate pens of 10 birds each. The positive control (PC) diet consisted of a standard corn and soybean meal formulation. The negative control (NC) diet was formulated with a 10% inclusion of wheat and 10% distillers dried grains with solubles (DDGS). This diet also contained 100 kcal/kg less metabolizable energy (ME) than the PC diet. Increasing inclusions of AC1 were applied to the NC diet to contain 50, 100, 200, and 400 U β-Glucanase (β-Glu-U) per kg of feed. Preliminary homogeneity in mash and pellet stability (80, 85, and 90°C pelleting) experiments demonstrated that AC1 was properly distributed during mixing (6% coefficient of variation) and was thermally stable (90% β-Glucanase activity following 90°C pelleting). Bird performance and digesta viscosity were measured on d14 and d21. Feed intake (FI) was similar across all treatments throughout the study ($P > 0.05$). Live weight gain (LWG) was the highest for PC fed birds from d1–14; however, birds fed NC with 400 β-Glu-U/kg had similar LWG as PC ($P > 0.05$). Day 1–21 feed conversion ratio (FCR) was the lowest for PC fed birds; however, birds fed NC with 400 β-Glu-U/kg had similar FCR as PC ($P > 0.05$). Birds fed the NC diet had lower LWG by 21g and higher viscosity by 0.51cP than the birds fed the PC diet on d14 ($P < 0.05$), but treatment differences were not significant on d21 ($P > 0.05$). Birds fed the NC diet with either 200 or 400 β-Glu-U/kg had similar d14 digesta viscosity as birds fed the PC diet ($P > 0.05$). These data indicate that NSP ingredients may have a greater impact on digesta viscosity early in broiler growth and that AC1 inclusion was efficacious to reduce viscosity and improve performance, particularly at higher doses (200 and/or 400 β-Glu-U/kg).

Key Words: broiler, NSP, viscosity, wheat, β-glucanase

133 Effects of xylanase and beta-glucanase application in corn-soy diets on performance and digestibility of male broilers fed diets with two different AME levels. K. Brown*[1], K. Smith[1], G. Gonzalez-Ortiz[2], and J. Lee[1], *[1]Texas A&M University, College Station, TX, [2]AB Vista, Marlborough, United Kingdom.*

The objective of the current experiment was to evaluate the inclusion of xylanase (16,000 BXU/kg) and β-glucanase (20,000 BU/kg), separately and in combination, in corn-soy based broiler diets with 2 AME levels (High and Low, with a 150 kcal/kg difference in all phases). Cobb 500 male broiler chicks (2,640) were placed in 8 experimental treatments, with a 2 (energy) × 2 (xylanase) × 2 (β-glucanase) factorial design. Each treatment had 11 pens with 30 chicks/pen. Starter (d 0–18), grower (d 18–34) and finisher (d 34–42) diets, and water were available *ad libitum*. On d 18, 34 and 42, ileal contents were collected for the determination of ileal digestible energy (IDE) and nitrogen (IDN). Statistical comparisons were performed using a 2 × 2 × 2 factorial ANOVA. Interactions, were determined by a one-way ANOVA and differences ($P \leq 0.05$) between individual treatment means were separated by Duncan's Multiple Range Test. Multiple interactions were observed between factors for BW and mortality corrected feed conversion ratio (FCR), mainly present between xylanase and β-glucanase inclusion. Inclusion of both enzymes in the High AME diet suppressed feed consumption and BW at 18 d of age, while no differences in main effect means or individual treatment means were observed on d 34 or 42. Reducing the AME level of the diet increased ($P < 0.001$) starter phase FCR. During the grower phase, xylanase improved ($P < 0.05$) FCR in the low AME diet but no improvements were observed in the High AME-fed broilers. Cumulative FCR (d 1 to 42) was elevated ($P < 0.05$) in the Low compared with the High AME-fed broilers. Inclusion of xylanase in the Low AME diet reduced ($P < 0.05$) overall FCR to levels similar to the High AME no-enzyme fed broilers. Xylanase and β-glucanase inclusion reduced FCR compared with their respective controls but no additional improvement was observed with their combination. Interactions were also observed on energy digestibility on d 18 and 34. On d 18, combined enzyme administration in the Low AME diet increased ($P < 0.05$) IDE while no increase was observed in the High AME diet when enzymes were fed. On d 34, individual inclusion of xylanase or β-glucanase increased ($P < 0.05$) IDE values in the Low AME diet however only dual administration increased ($P < 0.05$) IDE of the High AME diet. On d 18 and 34, IDN was increased ($P < 0.05$) with xylanase or β-glucanase inclusion. These data highlight the ability of xylanase to improve performance in corn/soy diets and suggest that additional exogenous enzyme inclusion may not be necessary to further increase the response.

Key Words: xylanase, β-glucanase, energy, broiler, performance

134 Effects of increasing levels of a multicomponent enzyme on male broiler growth performance and nutrient digestibility. T. Lester*[1], K. Brown[1], R. Poureslami[2], K. Bregendahl[2], and J. Lee[1], *[1]Texas A&M University, College Station, TX, [2]ADM Animal Nutrition, Decatur, IL.*

An experiment was conducted to evaluate the effects of increasing levels of a multi-component enzyme containing xylanase and cellulase (Empirical NSP, ADM Animal Nutrition) in reduced energy diets on male broiler growth performance and nutrient digestibility. Cobb 500 male broiler chicks were placed in 7 experimental treatments consisting of a positive control (PC), negative control (NC; -132kcal/kg of PC), and 5 treatments with increasing levels of the multi-component enzyme (990; 1,980; 2,970; 3,960; or 4,950 units of xylanase per kg of feed. Each experimental treatment had 12 replicate pens with 33 animals per replicate. Three dietary phases were fed during the trial: starter (d 0–14, crumble), grower (d 14–28, pellet), and finisher (d 28–41, pellet). Body weight and feed consumption measurements were taken at the conclusion of each dietary phase and were used to calculate mortality-corrected feed conversion ratio (FCR). On d 28, 5 broilers per replicate were randomly selected and ileal contents were collected for the determination of ileal digestible energy (IDE). Data were analyzed via a one-way ANOVA, and means deemed significantly different at $P \leq 0.05$ were separated using Duncan's Multiple Range Test. Linear and quadratic regression were used to determine enzymatic impact on performance and digestibility parameters. Body weight was not impacted by energy level between the PC and NC diets nor with the inclusion of multi-component enzyme

at any of the measured time points. The FCR was increased ($P < 0.05$) with the reduction of energy in the NC diet as compared the PC fed broilers in each of the 3 phases of production and cumulatively from d 1 to 42. Inclusion of a multi-component enzyme was beneficial to FCR, leading to a significant reduction ($P < 0.05$) in d 1 to 42 FCR in broilers fed all levels of the multi-component enzyme with exception of the highest level. The IDE was the highest for the PC fed broilers, as expected, and higher ($P < 0.05$) than NC fed broilers. Enzyme inclusion increased ($P < 0.05$) IDE value of the diet at all levels of inclusion compared with the NC broilers with exception of the highest inclusion rate (4,950 xylanase units per kg feed). Quadratic regression analysis indicated significant impacts of the multi-component enzyme on FCR and IDE with maximum response between 1,980 and 2,970 units of xylanase per kg of feed. These data demonstrate the benefits of a new multi-component enzyme containing xylanase and cellulase providing guidance on dosage rate.

Key Words: broiler, energy, xylanase, cellulase, digestibility

135 Efficacy of carbohydrase enzymes in diets varying in ingredient composition when fed to coccidiosis vaccinated broilers. B. Bodle*[1], M. Jackson[2], and S. Rochell[1], *[1]University of Arkansas, Fayetteville, AR, [2]Huvepharma Inc., Peachtree City, GA.*

An experiment was conducted to investigate the effects of 2 carbohydrase enzymes on growth performance, apparent ileal nutrient and energy digestibility (IDE), and processing characteristics in coccidiosis-vaccinated broilers fed diets containing animal protein meal (APM) or all vegetable-based (VEG) diets. Four APM or VEG diets included: 1) a positive control (PC), 2) a negative control (NC), 3) the NC diet + a multi-carbohydrase enzyme (HZY) (Hostazyme, Huvepharma, Inc.), and 3) the NC diet + a novel combination of HZY and an α-galactosidase (XAG) (Huvepharma, Inc.) for a total of 8 dietary treatments. Treatments were maintained across starter (0 to 14 d), grower (15 to 28 d), and finisher phases (29 to 42 d), and in each phase, negative control diets were formulated to contain 100 kcal/kg less apparent metabolizable energy than the PC diet. Animal protein-based diets contained 7.0% of an animal protein blend in the starter, 3.5% animal protein blend + 3.5% feather meal in the grower, and 2.5% animal protein blend + 2.5% feather meal in the finisher, and all diets within a phase were formulated to be isonitrogenous. Titanium dioxide was used as an indigestible marker for the determination of ileal digestibility. All birds received a 2x dose of a live coccidiosis vaccine (Advent, Huvepharma Inc.) by oral gavage immediately before placement. Eight replicate floor pens of 26 Cobb 500 male broilers per treatment were placed. Overall FCR (0 to 42 d) was lower ($P < 0.01$) for birds fed VEG diets than for birds fed APM diets, with no difference in BWG between these groups. Compared with birds fed the NC diets, birds fed the PC diets had lower ($P < 0.01$) overall FCR and similar BWG in both the APM and VEG groups. No effects ($P > 0.05$) of enzyme inclusion were observed on overall growth performance. Carcass and breast yields were higher ($P < 0.01$) for broilers fed VEG diets than for those fed APM diets, with no differences ($P > 0.05$) among birds fed PC, NC, or enzyme-containing diets. At 14 d, IDE was not different between APM and VEG-fed broilers, but was 128 and 161 kcal/kg lower in NC diets than in PC diets for the APM and VEG groups, respectively. Inclusion of HZY and XAG increased ($P < 0.05$) IDE of the APM NC diet by 178 and 90 kcal/kg, respectively, but did not influence ($P > 0.05$) IDE of the VEG NC diet. Inclusion of HZY and XAG also improved ($P < 0.05$) N digestibility of the APM NC diet, but not the VEG NC diet ($P > 0.05$). In conclusion, feeding VEG diets promoted superior growth performance and processing yields compared with APM-fed broilers. Although enzyme inclusion improved IDE and N digestibility of the APM diet, this did not translate to improved performance.

Key Words: carbohydrase, digestibility energy, animal protein, all-veg, broiler

136 Enhancing nutrient utilization of broiler chickens through supplemental enzymes. K. J. Bogota*[1], J. Wilson[2], A. Cowieson[3], and T. Woyengo[1], *[1]South Dakota State University, Brookings, SD, [2]DSM Nutritional Products, Belvidere, NJ, [3]DSM Nutritional Products, Kaiseraugst, Switzerland.*

The objective was to determine effects of adding phytase, amylase, and a cocktail of non-starch polysaccharide degrading enzymes (NSPase) individually or in combinations to corn-soybean meal-based diet for broilers on apparent ileal digestibility (AID) of nutrients and dietary AMEn value. Four hundred and 80 male broiler chicks were divided into 80 groups and fed 8 diets in a completely randomized design (10 groups/diet) from d 15 to d 21 of age. The diets were basal diet unsupplemented or supplemented with phytase (1,500 FTU/kg; Ronozyme HiPhos), amylase (80 KNU/kg, Ronozyme HiStarch) and NSPase (75 g/metric ton, Ronozyme Multigrain) individually or in all possible combinations. The basal diet contained the phytase at 1,000 FTU/kg, and was formulated to meet the NRC (1994) recommended nutrient requirements for broiler chickens except for ME, Ca and non-phytate P, which were reduced by 150 kcal/kg, 0.18%, and 0.15%, respectively. Data were subjected to ANOVA using the MIXED procedure (SAS Inst. Inc., Cary, NC) with cage as random term. Treatment means were separated by the probability of difference. Addition of phytase to the basal diet increased ($P < 0.05$) AID of P from 40.4 to 59.3%. Addition of amylase, NSPase or a combination of amylase and NSPase to the phytase-supplemented basal diet further increased ($P < 0.05$) AID of P to 63.4, 69.9 and 67.3%, respectively. Addition of phytase, amylase or a combination of amylase and NSPase to the basal diet did not affect dietary AMEn value. However, addition of NSPase alone or a combination of phytase and amylase or of phytase and NSPase to the basal diet improved ($P < 0.05$) dietary AMEn value from 3,203 to 3,339, 3,309 or 3,289 kcal/kg, respectively. In conclusion, it is more beneficial (with regard to AID of P and dietary AMEn) to add amylase and NSPase to phytase-supplemented diets for broilers. Because the basal diet contained phytase at 1,000 FTU/kg), the increase in AID of P due to supplemental phytase (1,500 FTU/kg) indicate that supplemental phytase at 2,500 FTU/kg is more beneficial with regard to improving AID of P than supplemental phytase at 1,000 FTU/kg.

Key Words: amylase, broiler, fiber-degrading enzyme, nutrient digestibility, phytase

137 Effects of calcium and phosphorus level in a diet containing phytase on male broiler performance and breast meat yield. M. Williams*[1], C. Coufal[1], K. Smith[1], P. Lessard[2], J. Broomhead[2], and J. Lee[1], *[1]Texas A&M University, College Station, TX, [2]Agrivida Inc., Woburn, MA.*

The objective of the current study was to evaluate the effects of calcium (Ca) and phosphorus (P) level in a diet containing super-dose levels of phytase (4 lb/ton GraINzyme; 4500 FTU/kg, analyzed in pelleted feed) on male broiler performance and breast meat yield. The experimental design consisted of a 3 (Ca reductions; 0.11, 0.13, and 0.15%) × 2 (P reductions; 0.12 and 0.15%) factorial yielding a total of 6 dietary treatments. Each treatment included 10 replicates with 21 Ross 708 male broilers placed per replicate (1260 broilers total). The dietary program

consisted of 4 dietary phases including a starter through d 14 (0.9% Ca; 0.45% aP), grower from d 15 to 28 (0.84% Ca; 0.42% aP), finisher from d 29 to 42 (0.76% Ca; 0.38% aP), and withdrawal from d 43 to 49 (0.76% Ca; 0.38% aP). Pen body weight (BW) and feed consumption (FC) were measured on days of dietary changes (d 14, 28, 42, and 49). On d 28, 3 birds per replicate were euthanized and the right tibia excised for determination of bone ash percent and weight. On d 50, following an 8 h feed withdrawal period, 3 birds per replicate were removed and processed for carcass, breast and tender weights and yield. Results showed that early FC and BW (d 14) increased ($P < 0.05$) as Ca level was reduced further from 0.11% to 0.15%. On d 28, the reduction of Ca by 0.13 or 0.15% increased BW ($P < 0.05$) compared with 0.11% reduction. A Ca by P interaction ($P < 0.05$) was observed in d 28 BW because in the higher Ca diet (−0.11%), reducing P by 0.15% increased BW but in the lower Ca diet (−0.15%), reducing P by 0.15% reduced BW. Increasing the reduction of P from 0.12% to 0.15% increased starter feed conversion ratio (FCR; $P < 0.05$). Calcium reductions from 0.11% to 0.13% improved FCR ($P < 0.05$) during the grower (1.442 vs 1.424) and finisher phase (1.663 vs 1.639), with further 0.15% Ca reduction being intermediate (1.432 and 1.655, respectively). The lowest cumulative FCR's (d 28, 42 and 49) were observed with 0.13% Ca or 0.12% P reductions with Ca by P interactions observed through d 28 and 42 ($P < 0.05$), resulting from increased FCR with 0.15% P reduction, but only in 0.15% Ca reduction. No impacts of mineral level were observed on processing weights or yields except for an increase ($P < 0.05$) in tenderloin weight with 0.15% P reduction. Birds fed −0.13% Ca and −0.15% P had lower d 28 percent tibia ash ($P < 0.05$) compared with other diets. These data illustrate the importance of utilizing the correct Ca and P matrix value to maximize the broiler performance.

Key Words: broiler, phytase, calcium, phosphorus

138 Effect of limestone particle size, available phosphorus, and phytase supplementation on live performance and apparent ileal digestibility of calcium, phosphorus, and amino acids of male broilers chickens. D. Joardar* and J. Brake, *North Carolina State University, Raleigh, NC.*

This experiment investigated the effects of limestone particle size, available phosphorus (AvP) and phytase on live performance, tibia bone ash, and apparent ileal digestibility of calcium (Ca), phosphorus (P), and amino acids of male broiler chickens in a floor pen study. Two particle sizes of limestone were defined as fine (~0.2 mm) and coarse (~0.9 mm) by using the US sieve method. The analyzed Ca concentration of both limestone sources was similar (~395 g/kg). Eight experimental diets were formulated using fine and coarse particle sizes of limestone, 0.3% and 0.45% levels of AvP, and 1000 and 2000 FYT levels of phytase in a 2 × 2 × 2 design. Each experimental diet was randomly allocated to 6 replicate pens with 16 birds per pen and fed consecutively starter (0–16 d) and grower (17–33 d) diets. Apparent ileal digestibility of Ca, P, and amino acids were determined at 33 d using titanium dioxide (5 g/kg) as an indigestible marker. Bone ash was also determined at 33 d. Dietary phytase supplementation of low AvP (0.3%) diets improved ($P \leq 0.05$) BW gain, feed intake, and FCR at 16 d. Subsequently, BW gain, feed intake, and bone ash were improved ($P \leq 0.05$) at 33 d by the phytase supplementation. Fine limestone improved ($P \leq 0.05$) FCR at 16 d while coarse limestone improved ($P \leq 0.05$) feed intake and digestibility of Ca, P, and amino acids at 33 d of age. Phytase supplementation of low-AvP diets improved live performance and bone ash, as expected. Fine limestone improved FCR in the starter phase but coarse limestone diets resulted in greater feed intake and improved digestibility of Ca, P and, AA at 33 d of age. These data suggest that particle size of dietary limestone should be an important consideration when formulating broiler diets

Key Words: amino acids, bone ash, broiler, limestone, digestibility

139 The long-term effects of dietary available phosphorus and calcium, and phytase supplementation on bone mineralization in laying hens. K. Pongmanee*[1], K. Nadeau[1], C. Wyatt[2], R. Van Wyhe[2], and D. Korver[1], *[1]University of Alberta, Edmonton, AB, Canada, [2]AB Vista, Plantation, FL.*

Phosphorus and calcium play important roles in laying hen bone health. There is a lack of information on bone mineralization in hens fed low available phosphorus (aP) and calcium diets, and supplemented with phytase in the long term. The objective was to determine the effect of dietary aP and Ca, and an enhanced *Escherichia coli* 6-phytase supplementation on bone characteristics from 0 to 74 wk of age. White egg layer pullets (n = 400) were obtained at 1 d of age, housed in one of 50 pullet-rearing cages (n = 8 per cage) and moved to individual cages (n = 50 to 52 per treatment) at 19 wk of age in a completely randomized design. Pullets were allocated to 5 dietary treatments: positive control (PC), a nutritionally complete corn-soy-canola-based diet phase-fed according to the primary breeder management guide; a negative control (NC) diet similar to the PC diet with reduced aP (by 0.15% of the diet) and Ca (by 0.16% of the diet); and the NC diet supplemented with either 300, 600 or 1,200 FTU phytase/kg. At each of 6, 15, 18, 42, 54, 64, and 74 wk of age, body weight (BW), feed intake (FI), and after 18 wk, egg production (EP) were measured. Femurs were excised post-mortem from 8 to 10 birds per treatment. The left distal femur was scanned using quantitative CT (QCT) to determine total, cortical, and trabecular bone mineral density (BMD) and bone cross-sectional area. Bone mineral content (BMC) was calculated by multiplying BMD by bone cross-sectional area. Bone breaking strength (BBS) and bone ash of the right femur were determined. Data were analyzed by 2-way ANOVA using the Mixed procedure for the main effects of diet and age, and their interaction. Correlation coefficients (r) among bone traits were measured. Differences were considered significant at $P \leq 0.05$. The NC birds had lower BW than the PC birds, but 1,200 FTU phytase/kg increased BW back to the level of the PC. Neither aP and Ca levels nor phytase affected FI or EP. Throughout the laying cycle, the NC+600 FTU phytase/kg tended to increase BBS ($P = 0.058$) and bone ash ($P = 0.062$). The NC diet reduced total BMD (404.34 ± 4.24 mg/cm^3) and cortical BMC (15.03 ± 0.15 mg/mm), but 600 FTU phytase/kg restored the total BMD (434.34 ± 7.68 mg/cm^3) and cortical BMC (16.84 ± 0.53 mg/mm). The r between BBS and total bone ash was low (0.36; $P < 0.001$), likely due to the influence of non-structural medullary bone, while r of BBS and cortical BMC was 0.69 ($P < 0.001$). The significant correlations between total bone ash and trabecular BMD, total BMD, and total BMC were 0.95, 0.92, and 0.88, respectively. Supplementation of 600 FTU phytase/kg allowed for reductions aP and Ca by 0.15% and 0.16% of the diet, respectively, and prevented bone loss in laying hens.

Key Words: phosphorus, phytase, bone mineralization, laying hen

140 Assessment of superdosing phytase on broiler phosphorus digestibility, ileal digestible energy and bone mineralization. J. Wang*[1], M. Coelho[2], A. Troescher[3], P. Ader[3], and W. Kim[1], *[1]University of Georgia, Athens, GA, [2]BASF Corporation, Humble, TX, [3]BASF, Lampertheim, Germany.*

We previously reported superdosing of phytase at 3000FTU/kg to a reduced calcium, available phosphorus (avP) and metabolizable energy

(ME) diet increased broiler performance (2018, IPPE). To gain a better knowledge of superdosing phytase effect on a nutrient deficient diet (reduced Ca, avP and ME), samples from previous experiment were further analyzed to evaluate the effect of superdosing phytase on phosphorus digestibility, ileal digestible energy (IDE) and bone ash. A total of 1,150 one-day old Cobb 500 male broilers were randomly allocated into 5 dietary treatments with 10 replicates and 23 birds each. Dietary treatments consisted of 1) positive control (PC) with all nutrients meet or exceed Cobb 500 nutrition manual; 2) negative control (NC) with 90 kcal/kg metabolizable energy (ME), 0.15% calcium and available phosphorus reduction from PC; 3). standard phytase dose control with 1,000 FTU/kg phytase addition on the top of NC (PC1); 4) reduced ME control with 200 kcal/kg ME reduction from NC (NC1); and 5) superdosing phytase with a dietary addition of 3,000 FTU/kg phytase on the top of reduced ME control (T1). The study was conducted as a completely randomized design and all data were analyzed by a one-way ANOVA. At d 28 and d 42, 5 birds from each replicate were sacrificed. Ileal digesta (d 28 and d 42) and left tibia (d 28) were collected for nutrient digestibility and bone ash. At d 28, addition of phytase at 1,000 FTU/kg to NC group and 3,000 FTU/kg to reduced ME group improved ($P < 0.05$) phosphorus digestibility from 50.3 and 59.9% to 79.1 and 78.8%, respectively. NC and reduced ME control group (NC1) decreased ($P < 0.05$) IDE compared with PC group, whereas the addition of phytase at 1,000 FTU/kg to NC group (PC1) and 3,000 FTU/kg to reduced ME group (T1) improved ($P < 0.05$) IDE to the same level with PC group. At d 42, reduced ME control group (NC1) showed the lowest IDE among all dietary treatments. Superdosing phytase at 3,000 FTU/kg (T1) increased ($P < 0.05$) IDE from 2,706.2 to 2,909.0 kcal/kg compared with reduced ME group (NC1) and it reached the same level with PC1 group (3,003.8 kcal/kg). However, it is still lower than the PC group (3102.1 kcal/kg). Bone ash results showed a reduction of calcium, avP and ME (NC and NC1) decreased ($P < 0.05$) the ash weight, ash percentage (ash weight/ fat free dry weight) and ash density (ash weight/ bone volume, g/ml). The addition of phytase at 1000 FTU/kg to NC group (PC1) and 3000 FTU/kg to reduced ME control group (T1) improved ($P < 0.05$) ash weight, ash percentage and ash density to the same level with PC group. In summary, superdosing phytase at 3,000 FTU/kg improved IDE, phosphorus digestibility, ash weight and ash percentage.

Key Words: phytase, digestibility, bone ash, broiler, superdosing

141 Influence of phytase addition in diets fed to broilers on plasma inositol over time and inositol phosphate ester concentrations at 28 days of age. R. Kriseldi*[1], C. Walk[2], J. Johnson[1], M. Bedford[2], and W. Dozier, III[1], *[1]Auburn University, Auburn, AL, [2]AB Vista, Marlborough, United Kingdom.*

An experiment was conducted to evaluate effects of supplementing broiler diets with phytase at 0, 400, and 1,200 phytase units (FTU)/kg on inositol phosphate (IP) esters in the gizzard and ileal digesta and plasma inositol concentrations over time at 28 d of age. Twenty 4 Ross × Ross 708 male chicks were placed in battery cages (4 birds per cage; 0.116 m^2 per bird) from 1 to 21 d of age. Broilers were caged individually from 22 to 28 d of age. Broilers received common starter (1 to 14 d of age) and grower (15 to 27 d of age) diets formulated to contain 1.23 and 1.10% digestible Lys and 3,053 and 3,086 kcal AME_n/kg, respectively. At 27 d of age, a catheter was placed in the brachial vein of the left wing of broilers to avoid repeated puncture of the vein during blood collection. Birds were provided a 15 h recovery period to become acclimated with the catheter. At 28 d of age, a baseline blood sample was collected from each bird before feeding experimental diets. Broilers received 1 of 3 experimental diets formulated to contain 0, 400, and 1,200 FTU/kg, respectively, in Diets 1, 2 and 3. Diet 1, the positive control, was formulated to contain 0.76% calcium and 0.36% non-phytate phosphorus, whereas calcium and non-phytate phosphorus concentrations in Diets 2 and 3 were 0.16 and 0.15% lower than Diet 1, respectively. Blood was collected from 20 to 240 min at 20-min intervals with a final blood collection at 480 min to determine plasma inositol concentrations. Following blood collection, birds were sacrificed and gizzard and ileal digesta were collected to evaluate IP ester destruction. Plasma inositol concentration of broilers was not different ($P = 0.34$) among the dietary treatments at each time point from 0 to 480 min. A cubic increase ($P = 0.023$) of plasma inositol concentration of broilers from 20 to 240 min was observed, regardless of the dietary treatments. The concentration of IP6 in broilers provided the 1,200 FTU/kg diet was 1/3 ($P < 0.01$) of the IP6 concentration in birds with 400 FTU/kg diet both in the gizzard (1,264 vs. 4,176 nmol/g) and ileal digesta (13,472 vs. 33,244 nmol/g). Similarly, the addition of 1,200 FTU/kg increased ($P < 0.01$) inositol concentrations in the gizzard and ileal digesta of broilers by 2.5 (2,703 vs. 1,071 nmol/g) and 3.5 (16,485 vs. 4,667 nmol/g) fold, respectively, compared with adding 400 FTU/kg. The reduction and increase of IP6 and inositol concentrations in the digesta of broilers demonstrated that phytase addition at 1,200 FTU/kg is more effective in destroying IP esters than adding 400 FTU/kg. However, these responses did not translate to increased plasma inositol concentrations, which warrants further investigation.

Key Words: broiler, phytase, inositol, inositol phosphate

142 Effects of meat and bone meal, phytase and antibiotics on hematological indices and bone integrity in broiler chickens during necrotic enteritis challenge. H. Zanu*, K. Gharib Naseri, T. T. H. Nguyen, N. K. Morgan, S. Wu, and R. A. Swick, *University of New Engand, Armidale, NSW, Australia.*

This study was designed to evaluate the effect of meat and bone meal (MBM), phytase (PHY) and antibiotics (AB) on bone integrity and hematological indices of chickens during necrotic enteritis (NE). Ross 308 male day-old chicks (n = 672) were fed 8 diets in a2 × 2 × 2 factorial arrangement of treatments. There were6 replicates per diet and 14 birds per replicate.Factors were: MBM (0 or 60 g/kg in S, 50 g/kg G/F), AB (0 or 100 mg/kg Zn bacitracinplus 60 mg/kg salinomycin) and PHY (Quantum Blue 5G; 500 (using matrix values) or 1500 FTU/kg (with 500 matrix values). Wheat – SBM based diets were fed to 42 d. All birds were challenged with 5000 unattenuated sporulated oocysts each of *E. acervulina*, and *E. maxima and* 2,500sporulated oocysts of *E. brunetti*(Bioproperties Pty Ltd.) on d 9, and 10^8cfu *C. perfringens* Strain EHE-NE18 (known to express NetB toxin) on d 14 and d 15. On d 16, blood samples were taken from 2 birds per pen. On d 42, femur, tibia and toe were excised from 2 birds per pen after euthanasia. Bone breaking strength (BS) was measured usingan Instron Universal texture analyzer. Data were subjected to 2-way ANOVA using Minitab 18.1. Means with significant differences were separated by Tukey's HSD test at a probability level of 0.05. Main effects for blood: none vs AB - erythrocytes (2.06 vs 2.21 10^9/ml), hemoglobin (11.22 vs 12.16 g/dl)and packed cell volume (22.12 vs 23.90 10^9/ml); 500 vs 1500 phytase white blood cells (60.03 vs 72.65 10^6/ml) with no interactions detected ($P > 0.05$).None vs MBM platelets (1.97 vs 1.02 10^6/ml) with an AB × PHY interaction detected ($P < 0.05$). Without AB, with PHY increased from 500 to 1500 there were increased platelets and with AB, additional PHY decreased platelets. Main effects for bone: none vs MBM, toe ash% (10.6 vs 11.4), tibia ash% (44.1 vs 45.7), tibia BS N (354 vs 415), femur ash% (42.4 vs 44.4), femur BS N (300 vs 336). Across MBM, AB × PHY interac-

tions were detected. The addition of AB increased toe ash% with 500 PHY but not with 1500 PHY ($P < 0.05$). Without AB, tibia wt % was increased when PHY was increased from 500 to 1500 but with AB there was no change ($P < 0.05$).These results show that birds given AB during necrotic enteritis had more erythrocytes with higher hemoglobin content and greater packed cell volume compared with those receiving no AB. The greater platelet count and tibia wt % in birds receiving high phytase without AB indicate benefits of high PHY on wound repair and bone mineralization during NE challenge. The addition of MBM improved many bone integrity parameters during NE challenge.

Key Words: meat and bone meal, phytase, hematology, bone, necrotic enteritis

143 Comparison of two net energy (NE) calculations for broilers fed an exogenous amylase and a composite enzyme. K. Hilton*, J. England, P. Maharjan, G. Mullenix, C. Coon, A. Beitia, and J. Weil, *University of Arkansas, Fayetteville, AR.*

A total of 612 male broilers were randomly allocated to 3 treatments according to a randomized complete block design experiment with 4 replicates per treatment. Each pen was provided feed and water ad libitum throughout the grow-out period. The treatments tested were: 1) Negative control – negative basal diet without enzymes (NC), 2) Negative control+ composite enzyme, 3) Negative control+ composite enzyme +amylase (recommended level). At ages 12, 26 and 48 average treatment BW was determined and birds were selected within one standard deviation of mean BW and moved to the respiratory chambers for heat production (HP) determination. Chicks were evaluated during the starter phase from 13 to 14d, grower 27–28d and finisher 50–51d of age. A total of 48, 20 and 8 total birds from each treatment were evaluated during the starter, grower and finisher phases, respectively. All chicks evaluated at different ages were obtained from the same flock at the same time. Birds were moved to the respiratory chambers 1d before evaluation for a period of adaptation. Heat production (HP) kcal = $3.872*VO_2$ (L/d) + 1.195 VCO_2 (L/d) (Farrell, 1974) was measured for 1d. After HP was measured, fasting heat production (FHP) was measured for 24h. Heat increment was determined (HI) = HP – FHP (Farrell, 1974). Body composition was measured on d7, 14, 21, 28, 35 and 49 by dual energy x-ray absorptiometry (DEXA) to determine net energy gain (NEg = protein grams × 5.66 + fat gain grams × 9.35). Data was analyzed using JMP Pro 13 (SAS, 2016). Two NE equations were compared, Classic NE (kcals) = ME-HI versus Arkansas NE(kcals) = NEg + NEm, where NEm (net energy maintenance) = HP – HI. Data was analyzed using JMP Pro 13 (SAS, 2016). NEg was significantly impacted by the addition of exogenous composite enzymes in the starter and grower phases ($P < 0.0002$), with NC+ composite enzyme producing 100 and 447 kcals/kg respectively, more than NC birds. Arkansas NE increased with the addition of composite enzyme compared with Arkansas NE for the NC diet alone. Arkansas NE recovered an average of 92% calories, with NC plus composite enzyme producing the highest NE value because Classic NE only recovered 84%. This experiment indicates the addition of exogenous composite enzymes increases the NEg of broilers. Furthermore, when NE is expressed as NEm + NEg more calorie recovery is possible, because more calorie value is given to body composition than calories lost as heat increment.

Key Words: enzyme, amylase, net energy, body composition

144 Effects of three different dietary oil sources added to broiler breeder diets and evaluated for hen and progeny performance. A. Beitia*[2], J. Caldas[1], K. Hilton[2], G. Mullenix[2], J. Weil[2], P. Maharjan[2], N. Suesuttajit[2], M. Schlumbohm[2], J. England[2], and C. Coon[2], *[1]Cobb-Vantress, Siloam Spring, AR, [2]University of Arkansas, Fayetteville, AR.*

Changes in available nutrient for the embryo has an effect on hatchability, chick quality, livability, and progeny performance (Wilson 1996). Post-hatch, the chick continues to rely on fatty acid metabolism until the intestinal digestion is fully functional (Vieira, 2007; Moran, 2007). The objective of the study was to evaluate the effects of 3 different oil sources added to feeds of parent stock hens' (PS) on egg composition, hatchability, hen and progeny performance. Additional research is needed to determine if feeding more oleic acid from canola oil or corn oil can improve hen and progeny performance from parent stock breeders. 1008 Pullets were reared in floor pens and later transferred to individual cages and light stimulated at 21 wks. From 1 to 21wk, pullets were fed to meet Cobb 500FF target body weight for parent stock pullets. All treatments were fed a common pullet starter 0 to 28d; pullet grower I 5 to 15wk; pullet grower II 16 to 24 wks. At 24wk, a total of 162 pullets were randomly assigned to 3 different dietary treatments consisting of 54 hens per treatment. The first treatment, which represented a standard broiler breeder diet, served as a control and contained poultry fat. The second treatment contained corn oil as the oil source, while the third treatment contained canola oil. Treatment 1, 2, and 3 were isocaloric diets with 2925 kcal/kg and a fat addition of 2.10%. Daily egg production was recorded and all eggs laid weighed twice a week. Egg composition was measured every 2 wk from 24 to 60 wk by GE Lunar Prodigy (dual-energy x-ray absorptiometry, DEXA). Hen performance, hatchability, and early progeny performance were determined at 29, 32, 39, and 49 weeks of age. An ANOVA analysis was performed using JMP Pro 13 (SAS, 2016). At 60 weeks of age, EHH was 167.88, 175.65, and 175.10 for hens on treatments 1, 2, and 3 respectively. Hens fed corn oil or canola oil laid 7.77 and 7.22 more eggs per hen housed through 60 weeks of age, respectively, compared with hens fed poultry fat. Hatchability was significantly higher from hens fed canola oil ($P = 0.0096$) across all hatches in comparison to poultry fat and corn oil. Red hock significantly decreased from hens fed corn oil at 32 and 49 wks ($P < 0.005$). Progeny hatch body weight was significantly higher from hens fed corn oil ($P < 0.0001$) at 32 and 39 weeks in comparison to poultry fat and canola oil. The amount of linoleate acid (C18:2) was significantly higher in eggs from hens fed corn oil ($P < 0.005$) at 29, 39, and 49 weeks. Results from this study showed hens fed corn and canola oil had an improvement in egg quantity, size, decreased red hocks at hatch, and 14d progeny performance at 29, 32, 39, and 49 wks of age.

Key Words: hen performance, progeny performance, broiler breeder, dietary oil

145 The effect of certain feed additives on live performence of antibiotic-free broilers. K. Chasteen*, K. Macklin, J. Hess, and D. Connor, *Auburn University, Auburn, AL.*

Due to consumer concerns, production of poultry without antibiotics has become a priority. Exploration of viable alternatives to antibiotics is an important part of today's poultry research. Though not as efficacious, several products have shown encouraging results in improving bird growth and yield. The objective of this study was to define the ability of 3 such products to influence live performance and selected processing characteristics. The effects of these products on 49-d live performance weight, AFCR and foot-pad lesions; as well as on selected processing parameters – WOG, breast and tender yields were observed. In this study, 4 treatments were utilized. Treatment A consisted only of a basal feed. Treatments B, C and D contained the basal diets with the inclusion of a commercially available feed additive. These consisted of microencapsulated secondary plant compounds (treatment B) or microencapsulated essential oils (treatments C and D). For the experiment, day old Ross 708 female chicks were randomly placed into 48 pens with 25 birds being placed into each pen. Dietary treatments were assigned using randomized block design. Birds were fed a commercial like 4 feed regimen with the treatments added at manufactures recommended level to all phases. The starter was fed from D0–14, grower 1 from D14–28, grower 2 from D28–42 and withdrawal from D42–49. On D49 the birds and feed were weighed, from this data feed conversion was calculated (feed:gain ratio). Paws were scored on d 49 utilizing a scale of 0–2, with 0 being no lesions, 1 being lesions less than 7mm, 2 being lesions being greater than 7mm. Throughout the study, mortality was recorded daily; using this information adjusted feed conversion ratio was calculated. Median weight birds were selected for processing and data collected for each as WOG, breast and tender yield. All data was analyzed with General Linear Model Procedure in SPSS. Percentage data (Mortality Breast, Tender) was arcsine transformed before analysis. If significant ($P < 0.05$), means were separated using Tukey HSD. For live production data there were no significant differences between the treatments, however there was a trend of treatment B birds being heavier with a lower AFCR. The processing data was not statically significant. The results of this experiment seem to indicate that feeding the product in treatment B tended to improve bird live performance.

Key Words: feed additive, broiler, processing, ABF

146 In ovo and in-feed probiotic supplementation promotes overall growth and muscle development in broiler chicken. M. S. Muyyarikkandy*[1], D. Kuttappan[1], M. Schesinger[1], E. Mathew[2], M. Darre[1], and M. A. Amalaradjou[1], *[1]University of Connecticut, Storrs, CT, [2]Kansas State University, Manhattan, KS.*

Increasing concerns over the emergence of antibiotic-resistant pathogens resulted in the FDA directive curbing the use of antibiotic growth promoters (AGPs) in food animals. This has led to a need for safe alternatives to AGPs in poultry production. In this regard, probiotic supplementation to chicks has been shown to improve performance in market birds. However, the period of embryonic growth and immediate post-hatch development account for almost half of the productive life of modern broilers. Furthermore, this developmental period is critical to attaining quality broiler performance at marketing. Therefore, the present study investigated the potential use of probiotics to promote embryonic development and post-hatch performance in broiler chicken. For the study, embryonated Ross 308 (N=440) eggs were sprayed with phosphate buffered saline (control) or probiotics {*Lactobacillus paracasei* DUP 13076 (LP), *L. rhamnosus* NRRL B 442 (LR), or probiotic cocktail (PC)} on days 0, 5, 10, 14, and 18 of incubation. The eggs were incubated in a Hovabator with automatic turning and embryos were sampled at regular intervals for growth and weight measurements. On day 18, eggs were set in the hatcher for 3 days. Following hatch, birds were raised on feed with or without probiotics (~9 log cfu/g of feed) until the end of the study (21 days post-hatch). Chicks were sacrificed

once weekly, and morphometric parameters were recorded. Additionally, breast muscle (pectoralis major) sections were collected from embryos and chicks for muscle fiber density and gene expression analysis. The experiments were set out as a completely randomized design with stratified sampling, and data were analyzed using Proc GLIMMIX and Proc PLM of SAS. Early and sustained supplementation of probiotics significantly improved embryonic growth and post-hatch performance in broilers ($P < 0.05$). Specifically, in ovo supplementation with LP, LR or PC significantly increased relative embryo weight and crown-rump length when compared to the control. For instance, spray application of LR on embryonated eggs was associated with a 4% increase in embryo weight relative to the control on d18 of incubation. Further, the improved embryonic growth was accompanied by a concomitant increase in body weight gain and FCR in chicks. Moreover, probiotic treatments improved muscle growth and development in embryos and chicks by significantly upregulating several myogenic factors such as *myoD, myoG, igfIR, myf5, pax3, pax7,* and *mrf4* ($P < 0.05$). Hence, the above-mentioned probiotics could potentially be supplemented in ovo and in-feed to enhance the overall growth and performance in broiler chicken.

Key Words: probiotic, in ovo, in-feed, growth promoter, broiler chicken

147 Evaluation of *Saccharomyces cerevisiae* products and Bacillus on Broiler Performance. Y. AL-Jumaa*, H. Leyva-Jimenez, A. Alsadwi, K. Gardner, R. Abdaljaleel, and C. Bailey, *Texas A&M University System, College Station, TX.*

Using feed additives is one path to enhance the poultry production and health. The objective of this study was to evaluate the effects of *Saccharomyces cerevisiae*yeast cell wall (YCW), yeast probiotic (YP), yeast culture (YC), and a *Bacillus* spp. (Bac.) probiotic on market age male broiler chicken performance. The hypothesis was these feed additives will enhance the production performance, intestinal villi health, and fecal texture in market age Cobb-700 broiler chickens. Only males were used in this study for more homogeneity. The study employed a control and 5 treatments which were added to the basal diet; YCW 250 ppm, YP 250 ppm, YC 1250 ppm, combination of YCW 125 ppm + *bacillus*2×10^5 cfu/g of feed, *Bacillus*2×10^6. The chickens and the feed were weighted on d 1, 21, 35, and 42 (trial termination) to evaluate the production performance. Mid-gut samples were collected on d 21 of age for histology. The samples were taken 3 cm after Meckel's diverticulum region. Fecal samples were collected on 14, 28, 41 to evaluate the dry matter percentage of feces. Collected data were analyzed as one-way ANOVA using the GLM procedure of SPSS.Significance was accepted at $P \leq 0.05$. At d 35 the results showed that there was improvement in phase feed to gain ratio in all treatments compared with the control group. There was significant improvement in phase feed to gain ratio in Bac., YCW+ Bac., and YP compared with the control group. The values were 1.9, 1.87, 1.87, and 1.98 respectively. There was significant improvement in cumulative feed conversion on d 35 in YCW+ Bac. and YP compared with the control group, the values were 1.66, 1.66, and 1.73 respectively. At d 42, the YP treatment showed numerically improvement in cumulative feed to gain and feed conversion ratio compared with the control group, the values were 1.79 and 1.84 for cumulative feed to gain, and 1.76 and 1.81 for cumulative feed conversion. There were no significant differences in dry matter percentage of the fecal samples; nor significant differences in villi height, crypt depth, or villi to crypt ratio. In conclusion, all treatments numerically improved the feed to gain ratio and feed conversion ratio but only the yeast probiotic significantly improved the cumulative feed to gain ratio and cumulative feed conversion. The values foe the cumulative feed to gain were 1.84, 1.81, 1.81, 1.79, 1.84, and 1,84 for YCW-250, Bacillus 2×10^6, YCW + *bacillus*, YP, YC, and control respectively. There were no significant differences in the percent dry matter of the fecal samples or intestinal histology.

Key Words: broiler, feed additive, probiotic, performance, histology

148 Broiler performance as affected by dietary hemp seed cake inclusion. C. Phillips*, A. Fahrenholz, C. Parkhurst, and C. Ashwell, *North Carolina State University, Raleigh, NC.*

The production of hemp products that have been selected for seed production is common in some parts of the world such as Canada and is becoming more prevalent in the US. The seeds from hemp are used to produce a valuable oil and the remaining byproduct is referred to as hemp seed cake (HSC), which has the potential to be used as a source of protein and energy in livestock. This study was designed to evaluate the inclusion of hemp seed cake (HSC) as an alternative feedstuff in poultry diets, primarily as a replacement for soybean meal, and the dietary effect HSC has on broiler performance. At placement birds were randomly assigned to 1 of 3 treatment diets that contained either 20% HSC, 10% HSC, or 0% HSC. HSC primarily replaced corn and SBM. A total of 360 male Ross 708 × YPM broiler chicks were weighed, tagged, and placed into 36 battery style cages with 10 chicks per cage (12 replicates/treatment). The birds were raised for 21d. To evaluate BW and FCR overtime, average BW by pen and feeder weight were weighed weekly. At 7, 14, and 21d, 2 birds were sampled from each of the 36 pens to measure the percent of bone-in breast muscle yield. Birds were given feed and water ad libitum. All data was analyzed using one-way ANOVA of JMP 13. BW was similar among all pens and treatment groups at placement. At 7 d, there was a numerical trend for increased BW as HSC inclusion increased with the 20% HSC birds weighing 133 g, the 10% HSC birds weighing 130 g, and the 0% HSC group weighing 125 g. At 14d the birds receiving the 20% HSC diet were significantly heavier ($P \leq 0.005$) than the control birds. At 14 d, mean BW for the 20%, 10%, and 0% HSC treatments were 435 g, 408 g, and 365 g, respectively. At 21d the trend continued with the 20% treatment group exhibiting increased BW compared with the control group. Mean BW for the 20%, 10% and control treatments at 21d were 853g, 815g, and 774g, respectively. Through 21 d, the 20% HSC treatment exhibited significantly increased FCR compared with the 0% HSC treatment with the 10% HSC treatment intermediate (20% HSC, 1.33 > 10% HSC, 1.27 > 0% HSC, 1.21). As mentioned above, percent bone-in breast muscle yield was measured. At 7 d, no difference in breast weight was observed. At 14d, significantly larger ($P \leq 0.01$) breasts were observed in the both the 20% HSC and 10% HSC treatment compared with the 0% HSC treatment. The 20% HSC treatment had a significantly larger breast than the control group at 21d. No differences in mortality were observed. In conclusion, HSC could be used as an alternative feedstuff in poultry diets that could result maintained or improved performance.

Key Words: hemp seed cake, performance, broiler

149 The effect of different inorganic feed phosphate on feed manufacture and pellet quality. A. Bergeron*[1], J. Boney[2], A. Mereu[4], and J. Moritz[3], *[1]Texas A&M University, Houma, LA, [2]Pennsylvania State University, State College, PA, [3]West Virginia University, Morgantown, WV, [4]Yara, Oslo, Norway.*

Diets with elevated levels of corn distillers dried grains and solubles (DDGS) are notoriously difficult to pellet due to their high fat and fiber content. The type of inorganic feed phosphate (IFP) used in diet formulations has been shown to affect feed manufacture and pellet quality. The objective of the current study was to evaluate the effects

of different IFPs on feed manufacture and pellet quality when used in diet formulations containing DDGS. Diet formulations were corn and soybean meal based containing 6% DDGS and formulated to contain 0.36% non-phytate phosphorus and 1.6:1 Ca:P ratio. The different IFPs evaluated were dicalcium phosphate (DCP, 18.5% P, 23% Ca; FP&S, USA), monodicalcium phosphate (MDP, 19% P, 16.5% Ca, 4.5% Na; Mosaic, USA), monocalcium phosphate (MCP, 22.7% P, 16% Ca; Yara, Norway), and defluorinated phosphate (DFP, 18% P, 30% Ca, 5.3% Na; Eurochem, Russia). All diets were batched and manufactured at the West Virginia University pilot feed mill. Soybean oil was used as the fat source with 0.5% added at the mixer. Mash diets were conditioned using a short-term conditioner (0.31 × 1.30 m, 10-s retention time) incorporating a constant temperature of 82.2°C. A consistent rate of feed was conveyed into the conditioner for all dietary treatments. Pellets were extruded using a 40-horsepower California pellet mill with a 4.7 × 38 mm pellet die. The study utilized a randomized complete block design. A 450 kg batch of feed was the experimental unit and all treatments were manufactured on 3 different days that served as blocks. The diet that included DFP decreased hot pellet temperature, motor amperage, and pellet durability compared with DCP and MCP diets ($P < 0.05$). The diet that included MCP increased hot pellet temperature but maintained similar motor amperage and production rate relative to diets that included DCP and MDP ($P < 0.05$). Pellet durability assayed with the New Holmen Pellet Tester showed that diets containing MCP or DCP were similar but increased relative to diets containing MDP and DFP ($P < 0.05$). Inorganic feed phosphate source had an effect on frictional force within the pellet die that influenced hot pellet temperature, motor amperage, production rate, and pellet quality.

Key Words: feed manufacture, inorganic feed phosphate, pellet quality, DDGS, conditioner

150 Diets that differ in inorganic feed phosphates will affect early broiler performance and tibia mineralization. A. Lamp*[1], A. Mereu[2], and J. Moritz[1], *[1]West Virginia University, Morgantown, WV, [2]Yara International, Oslo, Norway.*

Inorganic feed phosphates (IFPs) are used in broiler feed formulation to meet mineral requirements and to improve feed manufacture and pellet quality. Different IFPs vary in mineral content and digestibility that may dictate their dietary inclusion. The objective of the current study was to feed broilers diets that differed in IFPs but that were formulated to be either similar in dietary non-phytate phosphorus (nPP) and calcium to phosphorus ratio (Ca:P), total IFP mineral content, or calculated dietary ileal digestible phosphorus and assess early broiler performance and tibia mineralization. The different IFPs evaluated were dicalcium phosphate (DCP, 18.5% P, 23% Ca; FP&S, USA), monodicalcium phosphate (MDP, 19% P, 16.5% Ca, 4.5% Na; Mosaic, USA), monocalcium phosphate (MCP, 22.7% P, 16% Ca; Yara, Norway), and defluorinated phosphate (DFP, 18% P, 30% Ca, 5.3% Na; Eurochem, Russia). These IFPs were used in formulations with similar nPP (0.22%) and Ca:P (1.4:1). In addition to these 4 treatments, a mixture of MCP, monosodium phosphate, and DCP was used to assimilate total mineral content of MDP (Mixture). Moreover, a mixture of MCP and silicon dioxide was used to produce a similar calculated ileal digestible phosphorus as the DCP diet (digMCP). All diets contained phytase and were formulated to nPP levels below requirement to best demonstrate treatment differences. The study utilized a randomized complete block design with 10 replications of 10 male Ross × Ross 308 broilers fed 6 total dietary treatments. Linear contrasts were utilized to compare treatments of interest. Performance measurements were taken on D7, D14, and D21, and tibia mineralization was assessed on D21. Birds fed MCP demonstrated increased LWG at each measurement period (D7: 0.007kg, D14: 0.015kg, D21: 0.028kg) compared with DCP when diets were formulated to similar nPP and Ca:P ($P < 0.005$). Tibia ash percentage demonstrated a 1.5% increase for birds fed either MCP or MDP compared with birds fed DCP or DFP when diets were formulated to similar nPP and Ca:P ($P < 0.005$). Diets formulated with Mixture or digMCP were similar in all performance measurements ($P > 0.05$). However, MDP fed birds had a 1.05% increase in tibia ash percentage compared with birds fed Mixture ($P < 0.05$) but not to MCP. These data suggest that different IFPs may affect early broiler performance and phosphorus bioavailability differently when diets are formulated to similar nPP and Ca:P, as well as total IFP mineral content. Diets formulated using calculated dietary ileal digestible phosphorus may optimize feed P content.

Key Words: inorganic feed phosphate, monocalcium phosphate, dicalcium phosphate, monodicalcium phosphate, defluorinated phosphate

151 Green banana meal as prebiotics for broilers up to 21 days of age. E. Muro*[1], J. Sartori[1], J. V. Filho[1], T. Santos[1], L. Zanetti[1], G. Pasquali[1], R. F. Netto[1], J. Batistioli[1], L. Lopes[1], A. C. Neto[1], J. Denadai[1], D. Souza[1], R. G. A. C. Araujo[1], and A. C. Bolfarini[2], *[1]São Paulo State University (UNESP), Botucatu, Brazil, [2]School of Agronomic Sciences, Botucatu, Brazil.*

Using regionally adapted plant raw materials is fundamental to improve the supply of feed that can replace commonly used components in animal diets. Some meals, such as green banana meal (GBM), are known to have sugars with prebiotic potential. Therefore, 2 experiments were conducted to determine the energy value of GBM and performance of broiler chickens fed this product in initial phase of growth. In Experiment I an energy metabolizability trial was conducted, 60 male Cobb 500 broilers were randomly distributed in 2 treatments with 5 replications of 6 birds each: reference corn/soybean meal ration and test ration (20% of reference diet substituted for GBM). The apparent metabolizable energy corrected for nitrogen of the GBM was 1.421 kcal AME/kg, expressed as dry matter, characteristic of its composition in non-digestible sugars. A performance trial, up to 21 d of age was conducted in experiment II, 400 Cobb 500 males chickens were allotted in a completely randomized experimental design with 2 treatments [control with antibiotics (avilamycin20% at 50 ppm inclusion) and 0.2% inclusion of GBM without antibiotics] and 8 replicates of 25 birds each. The data collected were submitted to ANOVA followed by Tukey's means test at 5% of probability. GBM inclusion up to 21 d of age negatively affected ($P < 0.05$) the mean final weigh, mean weight gain and consequently average daily weight gain as well as feed conversion rate. Viability and feed intake did not differ significantly between treatments. The observed decrease in performance does not allow us to recommend green banana meal as a substitute for performance-enhancing antibiotics under these experimental conditions.

Key Words: functional sugar, *Musa* spp.

152 Evidence of horizontal genetic exchange of chromosomally encoded markers between *Campylobacter jejuni* cells contributing towards its genetic diversity. D. Samarth* and Y. M. Kwon, *University of Arkansas, Fayetteville, AR.*

Evidence of horizontal genetic exchange of chromosomally encoded markers between *Campylobacter jejuni* cells contributing toward its genetic diversity. Genetic diversity is considered an important characteristic feature of many bacterial populations. It provides a genome plasticity to a bacterial population and increase capacity for environmental adaptation. Many epidemiological studies provide us with the evidence of horizontal gene transfer contributing toward bacterial genomic diversity. *C. jejuni* is one of the bacteria exhibiting largely non clonal population structure. *C. jejuni* is the major cause of acute enteritis in the United States of America. Human campylobacteriosis is often self-limiting diarrhea, sometimes followed by severe post-infection complications such as Guillain-Barré syndrome. In the present study, we provide the evidence of horizontal genetic exchange of chromosomally encoded genetic markers between *C. jejuni* cells in biphasic Muller Hinton media. For this experiment, we constructed two *C. jejuni* mutants with different antibiotic markers viz. chloramphenicol and kanamycin resistance markers present at two different neutral genomic loci. The cultures of both mutants were mixed together in biphasic MH medium and incubated in microaerophilic conditions for 5 hrs. Appropriate serial dilutions were then plated on MH agar plates supplemented with both antibiotics and incubated for 2 days. The recombinant cells with double antibiotic markers were generated at the frequency of 0.0214% as compared to parental population. We further evaluated the effects of chicken cecal extracts on recombination efficiency using the same assay. The recombination efficiency was significantly increased (approximately 10 fold) in the presence of chicken cecal extracts in comparison to the biphasic MH media (control; $P < 0.05$). We also found that the recombination efficiency was significantly decreased (approximately 100 fold) by addition of DNase I to the assay in comparison to the control group (no DNase I; $P < 0.05$). Our finding indicates that horizontal gene transfer in *C. jejuni* cells can be facilitated in the gut environment contributing *in vivo* genomic diversity of *C. jejuni*, and is mediated by naked chromosomal DNA in the extracellular environment.

Key Words: *Campylobacter*, gene transfer, genetic diversity, chicken ceca, DNase I

153 The persistence of *Salmonella* after completion of sanitization procedures of a poultry processing plant. T. Obe*[1], R. Nannapaneni[2], W. Schilling[2], C. McDaniel[2], and A. Kiess[2], *[1]Mississippi State University, Starkville, MS, [2]Mississippi State University, Mississippi State, MS.*

Salmonella is a foodborne pathogen that is associated with poultry. Therefore controlling *Salmonella* during poultry processing is very important. Cleaning and sanitizing equipment surfaces before and after the processing of poultry meat is an important *Salmonella* control method. Therefore, the objective of this study was to evaluate the persistence of *Salmonella* on poultry processing equipment following cleaning and sanitation. A total of 19 locations within a commercial processing plant were sampled at 3 time points on 3 separate visits for a total of 171 samples. *Salmonella*-positive isolates were recovered from samples using the USDA MLG 4.09 conventional method. Presumptive *Salmonella* colonies were subjected to biochemical tests for confirmation. *Salmonella* isolates that were recovered after sanitization were tested for biofilm forming ability at 25°C using the crystal violet assay and minimum inhibitory concentration (MIC) of common sanitizers against the isolates were further examined following CLSI guidelines. RCB design was used for all experiments with a 3 × 19 factorial arrangement for prevalence. Means were separated using Fishers protected LSD when $P \leq 0.10$. For *Salmonella* prevalence within a processing plant, differences were observed over the 3 visits. As expected, there was a difference in the recovery of *Salmonella* due to the time of sampling defined as after processing (A), cleaning (B) and sanitization (C). *Salmonella* prevalence at time A (56%) was significantly higher, while there was no difference between time B (26%) and C (14%). There was a location effect for the prevalence of *Salmonella* with the head puller having a significantly higher prevalence when compared with several other locations. There was a difference in the biofilms formed within locations where *Salmonella* was recovered. *Salmonella* that was isolated from the head puller produced significantly more biofilm than isolates from other locations. However, the majority of the recovered isolates possessed a moderate biofilm adherent ability. Furthermore, there was no significant difference in the MIC of peracetic acid (PAA) and quaternary ammonium compound (QAC) against the *Salmonella* isolates recovered from different locations within the plant. However, there was a negative correlation between the MIC of QAC and biofilm forming ability of the isolates ($P = 0.011$, $r = -0.79$). This suggests that the recovered isolates that do not adapt to QAC as planktonic cells may form stronger biofilms. These preliminary results indicate the established *Salmonella* prevalence and persistence within a poultry processing plant, but future studies are necessary to observe the extent to which this data applies to multiple plants.

Key Words: *Salmonella*, persistence, sanitization, quaternary ammonium compound (QAC), poultry plant

154 Comparison of sites colonized in broilers when challenged with *Salmonella* Enteritidis. L. Brooks*[1], J. Krehling[1], K. Chasteen[1], B. Singh[1], and K. Macklin[2], *[1]Auburn University, Lake City, FL, [2]Auburn University, Auburn, AL.*

Salmonella Enteritidis (SE) is one of the major bacterial foodborne pathogens known to cause illness and possibly death in humans. Poultry feeds can serve as potential sources of SE contamination either from use of contaminated ingredients or the environment within the feed mill. In growout facilities, litter contaminated with SE can be ingested, thereby serving as a potential source of colonization. Two studies were conducted to determine which tissues are colonized after inoculation either orally or via feed. For each study, 125 straight run chicks were obtained from a commercial hatchery on d 0 and 25 chicks were randomly assigned into 5 pens. These birds were fed a standard diet ad libitum, unless otherwise noted. The birds involved with the orally inoculated study were challenged on d 14 with 500µL of $1x10^4$ cfu of SE. For the feed inoculation study, on d 14, pens were each given access to 15.87 kg of $1x10^4$ cfu/g of SE contaminated feed, which was consumed in its entirety (~8d) before being put back onto non-contaminated feed. Both studies were terminated between d 35–37. The following 10 samples were collected from ~100 randomly selected birds and placed in buffered peptone water (BPW): breast, ceca, crop, kidney, neck skin, spinal cord, thigh, trachea and 2 pooled samples: bursa/thymus and liver/spleen.

Additionally, swab samples were collected from: abdominal cavity, bone marrow, cloaca, and lung. Following necropsy, samples were incubated in BPW overnight at 37C and then transferred to tetrathionate broth tubes and incubated for an additional 48–72h at 37C. All samples were plated onto XLT 4 agar containing naladixic acid 100 ppb and novobiocin 15 ppb and then incubated for 48h at 37C to determine *Salmonella* presence. Overall, birds challenged by oral gavage were 48% positive for SE, while birds challenged via feed were 66% positive. Data was analyzed using a *t*-test ($P \leq 0.05$) to compare incidences based on the inoculation route. From all the samples harvested, the oral challenge of SE (10.7%) was lower than the feed challenge (13.0%); however it was not significant. Samples found to be significantly different based on the route of inoculation include cloaca, ceca, liver/spleen, and trachea. Differences between both inoculations can be attributed to the ~8d exposure of SE in the feed as opposed to a one-time oral gavage at d 14. Based on this study, the route of inoculation could have an impact on the incidence in which SE colonizes different tissues. Future studies will continue to evaluate other routes of inoculation to determine if a correlation can be made.

Key Words: challenge, *Salmonella* Enteritidis, colonize, oral gavage, feed

155 Comparative genetic profiles and antimicrobial susceptibility of *Salmonella* isolated from broilers and broiler breeders on a poultry research farm. G. Walker*[2], M. Suyemoto[1], L. Borst[1], and J. Brake[2], [1]*College of Veterinary Medicine, North Carolina State University Raleigh, NC,* [2]*North Carolina State University, Raleigh, NC.*

Salmonella prevalence in live poultry and their housing environment is a concern due to associated food safety risks. Concurrent development of antimicrobial resistance and environmental persistence make eradication of the pathogen difficult. The objective of the current investigation was to evaluate the relatedness and antimicrobial resistance of *Salmonella* isolated from poultry in an integrated poultry research and production facility. One broiler breeder flock (BBF1) and 2 broiler flocks (BF1 and BF2) were reared over a 1.75-yr period. Hatching eggs were obtained from BBF1 to produce BF1 chicks, while BF2 chicks were progeny of a separate broiler breeder flock. BF1 and BF2 were reared 6 mo apart but in the same housing facilities, with routine cleaning and disinfection between the flocks. Duplicate *Salmonella* isolates were collected via litter sock sampling (BF1), cecal excision (BF1 and BF2), or cloacal swab methods (BBF1) and preserved for further evaluation. Serotyping revealed *Salmonella* Altona in BBF1 and *Salmonella* Senftenberg in BF1 and BF2. Genotypic characterization was achieved with $(GTG)_5$-PCR, which revealed sequence homology between *S*. Senftenberg isolates from BF1 and BF2. Each isolate was subjected to the broth microdilution method for minimum inhibitory concentration (MIC) determination of 27 antimicrobial agents. These agents were delivered with 2 unique 96-well plates (Sensititer) with formularies specific to both antimicrobials utilized in poultry production facilities and those employed to control gram-negative pathogens monitored by the National Antimicrobial Resistance Monitoring System. Isolates from the 3 flocks were resistant to clindamycin, erythromycin, novobiocin, penicillin, and tylosin tartrate while each demonstrated intermediate resistance to florfenicol and spectinomycin. These data demonstrated that *S.* Altona and Senftenberg were harbored by poultry, the latter persisted in broiler flocks, and both shared similar patterns of antimicrobial resistance in an integrated research operation.

Key Words: *Salmonella*, antimicrobial resistance, broiler, broiler breeder

156 The impact of cage-free housing system on horizontal transmission of *Salmonella* in eggs. G. Ward*[1], D. Jones[2], R. Gast[2], K. Anderson[3], M. Singh[1], and H. Thippareddi[1], [1]*University of Georgia, Athens, GA,* [2]*USDA ARS, Athens, Georgia,* [3]*North Carolina State University, Raleigh, NC.*

The current study evaluated the ability of *Salmonella* to penetrate nest run cage-free eggs via 2 inoculation methods. Nest box eggs were collected from a flock of cage-free layers (34 - 49 wks of age) with a stocking density of 0.121 m^2. Two day-old nest run eggs (n = 720; stored at 4°C) were utilized. Four nalidixic (200-ppm) acid-resistant *Salmonella* serovars: *S.* Enteritidis (SE), *S.* Heidelberg (SH), *S.* Kentucky (SK), and *S.* Typhimurium (ST) were used in this study based on links to foodborne illness and/or frequent production environment recovery from previous research. The trial included 2 inoculation methods (fecal paste and dip) with an approximate target concentration of 5 $\log_{10}$ cfu per g or mL to evaluate penetration of serovars. Inoculated eggs were placed in 4°C storage. Weekly sampling was conducted for 5 wks with each egg sampled at 3 locations to determine shell penetration: shell rinse, shell and membrane emulsion, and egg contents. Statistical analysis was conducted using Pearson's Chi Square test for independence. While no *Salmonella* spp. were detected in the egg contents, prevalence in the shell emulsion, across all serovars, diminished over time ($P < 0.05$). Differences in *Salmonella* penetration varied between serovars and inoculation treatments, where SE had higher prevalence on the shell in fecal inoculated (91.27%) than dip inoculated eggs (82.24%; $P < 0.05$). However, prevalence in the shell emulsion was not different between the 2 inoculation treatments for SE. ST prevalence was higher on the shell for dip inoculated (100%) versus fecal inoculated eggs (84.26%; $P < 0.05$). ST prevalence for shell emulsion was greater for dip inoculated (43.52%) versus fecal inoculated eggs (27.78%; $P < 0.05$). There was no significant difference in the prevalence of SH between inoculation treatments; respectively both treatments had prevalence levels higher than 88% throughout the duration of the study for shell rinse. SH resulted in a greater prevalence in the shell emulsions of fecal inoculated (37.14%) versus dip inoculated eggs (14.81%; $P < 0.05$). Finally, there was no difference in the prevalence of SK between inoculation treatments for both the shell rinse and the shell emulsion. Nest run cage-free eggs shell penetration by *Salmonella* spp. can occur throughout refrigerated storage. The contribution of fecal contamination on the surface of shell eggs to *Salmonella* spp. penetration should be taken into consideration when storing eggs before washing.

Key Words: *Salmonella*, eggshell penetration, cage-free, fecal contamination

157 Combined antimicrobial effect of conjugated linoleic acid overproducing *Lactobacillus* and berry pomace phenolic extracts on *Campylobacter jejuni*. Z. Tabashsum*, M. Peng, E. Kahan, and D. Biswas, *University of Maryland, College Park, MD.*

Campylobacter jejuni (CJ) is one of the prominent causes of acute gastroenteritis in the US and occurs through consumption of raw and undercooked poultry. Probiotics specifically genetically engineered *Lactobacillus casei* (LC-CLA) with increased ability to produce bioactive compounds such as bioactive conjugated linoleic acid (CLA) can act as antimicrobial against CJ along with other beneficial effects on host health. Additionally, prebiotics such as bioactive phenolic extracts from berry pomaces/byproducts can inhibit the enteric bacterial pathogens. In this study, we aim to enhance the efficiency of antimicrobial/beneficial activities and/or amount of production of bioactive compounds by combining berry pomace extracts (BPE) and conjugated linoleic acid (CLA)

overproducing *Lactobacillus*. We have generated genetically modified LC-CLA by overexpressing linoleate isomerase gene and evaluate the combined effect of BPE and LC-CLA against CJ. CJ growth pattern and inhibition in the presence of LC-CLA/BPE, CJ cell surface hydrophobicity, and auto-aggregation capability were determined. Adhesion and invasiveness abilities of CJ were carried out using cell-culture model. Expression of virulence genes of CJ was also compared. Significance in treatment difference was determined by ANOVA. In mixed culture condition, LC-CLA and CJ in the presence of 0.3mg/mL BPE, growth of CJ was reduced > 2.5 logs within 48 h ($P < 0.05$). Cell-free cultural supernatant (CFCS) of LC-CLA (CFCS-LC-CLA) with BPE (0.3mg/mL) showed > 3.0 logs cfu/mL ($P < 0.05$) reduction for CJ within 24 h. Treatments with CFCS also changed the cell surface hydrophobicity, auto-aggregation capacity of CJ, significantly. Interaction of CJ with cultured chicken fibroblast cells (DF1) were altered significantly ($P < 0.05$); the combined treatment reduced adhesion and invasion into DF1 cells by > 2.0 logs and > 1.5 logs, respectively. Further, combined treatment (BPE and LC-CLA) significantly ($P < 0.05$) altered the expression of multiple virulence genes such as *cadF*, *cdtB*, *ciaB*, *flaA*, *flaB* of CJ. BPEs enhance the effect of LC-CLA in reduction of CJ survival ability, host cell-pathogen interactions and virulence gene expression that may able to prevent colonization in poultry and reduce cross-contamination in poultry products and control foodborne infections with CJ in human.

Key Words: *Campylobacter jejuni*, *Lactobacillus casei*, berry pomace extract, food safety, poultry

158 Phytochemicals modulate *Campylobacter jejuni* proteome critical for biofilm formation. B. R. Wagle*[1], A. Upadhyay[1], I. Upadhyaya[1], K. Arsi[1], S. Shrestha[1], R. Liyanage[1], K. Venkitanarayanan[2], A. Donoghue[3], and D. Donoghue[1], *[1]University of Arkansas, Fayetteville, AR, [2]University of Connecticut, Storrs, CT, [3]USDA-ARS, Fayetteville, AR.*

Campylobacter jejuni is the leading cause of human foodborne illness globally, and is strongly linked with the consumption of contaminated poultry products. Several recent investigations have highlighted the role of *C. jejuni* biofilms in the environmental persistence of this pathogen. However, little is known about the proteome profile of *C. jejuni* during this physiological state. The objective of this study was to investigate the proteome of *C. jejuni* in biofilms or the free living planktonic state. In addition, the effect of 3 GRAS status phytochemicals namely, trans-cinnamaldehyde (TC), eugenol (EG) and carvacrol (CR) on the proteome of *C. jejuni* present in biofilms was evaluated. *C. jejuni* (strain NCTC 11168) biofilm was developed on polystyrene plates (37°C for 48 h) either in the presence or absence of sub-inhibitory concentrations of phytochemicals (0.01% TC, 0.01% EG or 0.002% CR). Proteins were extracted from biofilm using bacterial protein extraction reagent, and subjected to SDS-PAGE followed by in-gel tryptic digestion and LC-MS/MS based protein quantification. Similar methods were used for studying the proteome from planktonic cells. The experiment was conducted twice with triplicate samples. Data were analyzed using Scaffold Proteomic software. A total of 100 proteins were identified. The expression of 27 proteins was significantly modulated (fold change ~4.6 to 20) in the biofilms compared with planktonic cells ($P < 0.05$). These proteins contribute to cellular and metabolic process (NapA, NapB, HydB, RplL, RplJ, Tsf, SucD, AcnB, Tig, FrdA), biological regulations (Tpx, Trx) and membrane integrity (CadF, YajC, NrfA, Omp18, PetC, PorA, FliL, and Peb1A). The phytochemicals TC, EG and CR significantly downregulated NapA (required for signaling pathway during oxidative stress and significant for biofilm formation). In addition, TC and CR also reduced the expression of DnaK (chaperone protein) and bacterioferritin required for biofilm formation. The results suggest that a subset of *C. jejuni* proteome changes during biofilm formation, and phytochemicals modulate key proteins contributing to biofilm formation in *C. jejuni.*

Key Words: *C. jejuni*, biofilm, planktonic cell, phytochemical, proteomics

159 Inhibiting horizontal transfer of antibiotic resistance gene between *Salmonella* Heidelberg and *E. coli* by phytochemicals. P. Vinayamohan*[1], A. Pellissery[1], M. Muyyarikkandy[1], M. S. Nair[3], M. Darre[1], A. Donoghue[2], and K. Venkitanarayanan[1], *[1]University of Connecticut, Storrs, CT, [2]USDA, Fayetteville, AR, [3]University of Minnesota Twin Cities, Minneapolis, MN.*

Antibiotic resistance (AR) has emerged as a global public health threat. Bacteria have the ability to share genetic information, resulting in AR spread in a variety of environments, including manure and water. Horizontal gene transfer (HGT) represents the major route for AR spread in bacteria with conjugation constituting a common means for dissemination of AR genes. With food animals, including poultry being recognized as a reservoir of AR bacteria, poultry products represent a potential source of AR bacteria. Strategies that inhibit HGT between AR pathogens and commensal bacteria could potentially reduce the dissemination of AR genes in the environment and foods. *Salmonella* Heidelberg (SH) is a major AR foodborne pathogen associated with poultry. This study investigated the efficacy of 4 phytochemicals, namely caprylic acid (CA), carvacrol (CR), trans-cinnamaldehyde (TC) and β resorcylic acid (BR) in reducing β-lactamase gene (*blaTEM*, ampicillin resistance) transfer between *S.* Heidelberg and non-pathogenic *Escherichia coli* in Luria- Bertani broth (LBB), poultry manure and water. S. Heidelberg (bla_{TEM}+ve) and nalidixic acid (NA)-resistance induced *E. coli* were inoculated in 1:1 ratio in LBB, fresh poultry manure, and water in the absence and presence of sub-MIC (0.75 × MIC) of CA (0.38 mM), CR (0.16 mM), TC (0.13 mM) and BR (0.75 mM), and incubated at 37°C for 24 h. Transconjugant colonies were recovered by plating on tryptic soy agar (TSA) containing ampicillin and NA, donor cells (SH) were recovered on TSA+ampicillin, and recipients (*E. coli*) were enumerated on TSA+NA plates. The HGT of AR was calculated as the ratio of the number of trans-conjugants to the number of recipient cells. Additionally, trans-conjugant bacterial DNA was examined for *bla*TEM using colony PCR. The effect of phytochemicals on genes critical for conjugation in SH was determined by RT-qPCR. Duplicate samples of each treatment were included, and the study was replicated thrice. The data were analyzed using Proc GLM of SAS. All 4 phytochemicals reduced HGT frequency of bla_{TEM} gene between MDR *Salmonella* and *E.coli* ($P < 0.05$). TC, CR, CA and BR decreased HGT frequency by 70%, 85%, 93%, and 95%, respectively compared with the control. Colony PCR detected *bla*TEM in transconjugant *E. coli* populations confirming HGT occurrence. RT-qPCR results revealed that the phytochemicals upregulated (1.5 to 4.75 fold) the *finO* (repressor of conjugation), and decreased (1 to 4 fold) the transcription of *tra* genes (responsible for direct cell to cell contact) compared with the control ($P < 0.05$). Results suggest the potential use of phytochemicals for controlling AR spread in poultry manure and water, thereby improving food safety.

Key Words: antibiotic resistance, poultry, phytochemical, horizontal gene transfer, *Salmonella*

160 Efficacy of peracetic acid and zinc in reducing *Campylobacter jejuni* on chicken skin. S. Shrestha*[1], B. R. Wagle[1], A. Upadhyay[1], K. Arsi[1], I. Upadhyaya[1], A. Donoghue[2], and D. Donoghue[1], *[1]University of Arkansas, Fayetteville, AR, [2]USDA-ARS, Fayetteville, AR.*

Campylobacter jejuni is a leading cause of bacterial foodborne disease in humans worldwide, largely associated with the consumption of contaminated poultry products. With increasing consumer demand for natural and minimally processed foods, the use of Generally Recognized as Safe status antimicrobials is gaining attention for improving safety of poultry products. Peracetic acid (PAA; 220 ppm) is extensively used as an antimicrobial treatment in the US commercial poultry. However, approved dose of PAA produces inconsistent/low pathogen reductions. Metal ions have been used as effective antimicrobials for centuries. Zinc (Zn) is a GRAS status metal used in meat, fish packaging to prevent microbial spoilage. However, the efficacy of Zn for poultry carcass disinfection has not been investigated. The present study evaluated the efficacy of PAA either alone or in combination with Zn ($ZnSO_4.H_2O$) as an antimicrobial dip treatment in simulated chiller for reducing *C. jejuni* and *Pseudomonas* spp. (an important meat spoilage bacteria) on chicken skin. Two replicate trials were conducted. In each trial, 130 skin samples (4 cm × 4 cm each) were randomly divided into 13 treatments including baseline (inoculated samples but not subjected to dip treatments), PAA (0, 55, 110 or 220 ppm), Zn (0, 62.5 or 125 ppm) or combinations. Each skin was inoculated with a cocktail of 4 wild strains of *C. jejuni* (~7.4 log cfu/skin). Following 30 min of attachment, the samples were pre-chilled (15°C, 15 min) followed by 1.5 h of dipping in aforementioned treatments at 4°C (chilling simulation). Treated skins were drip dried (2 min) and sampled at 0 and 24 h of refrigerated storage for *C. jejuni* and *Pseudomonas* spp. counts (n = 5 skin/treatment/time point). The data were analyzed by ANOVA using MIXED procedure of SAS 9.3 and expressed as LSMEANS with differences considered significant at $P < 0.05$. Bacterial counts recovered from control was ~6 log cfu/sample for *C. jejuni* and ~4 log cfu/sample for *Pseudomonas* spp. The highest dose of PAA (220 ppm) reduced counts by ~0.5 - 1 log cfu/sample for both pathogens. Zn (125 ppm) reduced *C. jejuni* counts by ~1 – 1.5 log cfu/sample, however, no reduction was observed for *Pseudomonas* spp. compared with control. Select combination treatments synergistically reduced *C. jejuni* at 24 h of storage when compared with individual treatments. The combination of 220 ppm PAA with 125 ppm Zn was the most effective treatment and reduced *C. jejuni* by ~3.5 log cfu/sample and *Pseudomonas* spp. by ~2 log cfu/sample. Results suggest that combination of Zn and PAA could be a good strategy to control *C. jejuni* in post-harvest poultry.

Key Words: *C. jejuni*, peracetic acid, zinc, post-harvest poultry

161 Feed manufacture and *Salmonella* surrogate mitigation differences between standard pelleting and more thermally aggressive pelleting utilizing a hygienizer. T. Boltz*[1], J. Boney[2], and J. Moritz[1], *[1]West Virginia University, Morgantown, WV, [2]Pennsylvania State University, State College, PA.*

Feed hygienics are of ever increasing importance in providing safe feed to animals, and ultimately consumers. More thermally aggressive conditioning and pelleting techniques have been suggested to improve the mitigation of pathogens in feed. High conditioning temperature, long conditioning time, and hygienizers have been suggested to decrease *Salmonella* potentially associated with mash feeds. Hygienizers incorporate jacketed heat that enables feed to maintain a desired temperature for an extended period of time. The objective of the current study was to compare feed manufacture and *Salmonella* surrogate mitigation differences between standard pelleting and thermally more aggressive pelleting utilizing a hygienizer. Corn and soybean meal based mash feed was inoculated with *Enterococcus faecium* 8459, an appropriate nonpathogenic surrogate for *Salmonella*. Diets were either pelleted using standard practices that included 15 s conditioning at 70°C (Trt 1), or more thermally aggressive pelleting that included 30 s conditioning at 80°C and utilization of a hygienizer (Trt 2). Hygienizer temperature averaged 83°C for a 45 s duration. All conditioned feed was extruded through a 4.8 × 38 mm pellet die. Feed manufacture variables were recorded during conditioning and pelleting. Mash samples were collected before feed manufacture and pellet samples were collected directly after extrusion and cooled. All samples were flash frozen in liquid nitrogen and stored at −80°C before culture-based analysis. Treatments were replicated 3 times across 3 d of feed manufacture. Data were analyzed using a randomized complete block design. More thermally aggressive pelleting decreased pellet mill motor load ($P = 0.02$), and increased hot pellet temperature ($P = 0.02$). More thermally aggressive pelleting also tended to increase pellet durability ($P = 0.07$). *E. faecium* 8459 colonies were reduced with standard pelleting relative to inoculated mash and reduced further with more thermally aggressive pelleting ($P < 0.05$). Standard pelleting and more thermally aggressive pelleting resulted in a 3 and 4 log reduction in *E. faecium* 8459 colonies respectively, relative to inoculated mash. Thermally aggressive conditioning and pelleting utilizing a hygienizer may improve manufacture efficiency, pellet quality, and *Salmonella* surrogate mitigation.

Key Words: hygienizer, *Salmonella*, mitigation, pelleting, feed

162 In vitro evaluation of six commercially available *Bacillus* based probiotic supplements and their effects on an antibiotic resistant strain of *Clostridium bifermentens*. J. Sousa*[1], C. Castaneda[1], O. Gutierrez[2], C. McDaniel[1], and A. Kiess[1], *[1]Mississippi State University, Mississippi State, MS, [2]Huvepharma Inc., Peachtree City, GA.*

Necrotic enteritis is an enteric disease caused by *Clostridium perfringens*, which costs the poultry industry billions of dollars worldwide every year. As consumer trends have begun to favor antibiotic free and no antibiotics ever programs, it has become necessary to investigate the efficacy of antibiotic alternatives in an attempt to reduce pathogens like *Clostridium*. Therefore, the objective of this study was to evaluate 6 commercially available *Bacillus* based probiotic products for their ability to reduce different concentrations of an antibiotic resistant strain of *Clostridium bifermentens* (CB), in vitro. Treatments included the combination of an antibiotic resistant strain of CB, at 10^5, 10^4, and 10^3 cfu/mL with 6 commercially available *Bacillus* based probiotic products (P1-P6). For CB, a 24 h stock was diluted to create the desired concentrations. For each product, a 24 h stock was diluted to obtain 10^6 cfu/mL. For combination treatments, 1 mL of CB at the desired concentration was added to 9 mL of nutrient broth containing 10^6 cfu of probiotics and incubated anaerobically at 37°C. Afterward, 100 µL of each treatment was serially diluted at 0, 4, and 8 h of incubation, spread plated on their appropriate agar, and incubated at 37°C for 24 h under either aerobic or anaerobic conditions. Counts were log-transformed and analyzed using a factorial arrangement of treatments within a split plot design over incubation time. Data indicated that at 0 h of growth only P4 was able to reduce CB at the 10^3 cfu/mL concentration ($P < 0.05$). At 4 h of growth, certain products were able to reduce CB but it was dependent on the starting concentration of CB; 4 products reduced CB at the 10^3 cfu/mL concentration (P3, P4, P5, P6) ($P < 0.05$), 2 products reduced CB at the 10^4 cfu/mL concentration (P3 and P6) ($P < 0.05$), and 1 product reduced CB at 10^5 cfu/mL concentration (P1) ($P < 0.05$). At 8 h of growth, all

concentrations of CB were reduced by all products. For both 10^3 and 10^4 cfu/mL concentrations of CB, P1 and P3 reduced CB even further than P2, P4, P5, and P6 ($P < 0.05$); at the 10^5 cfu/mL concentration of CB, only P1 was able to further reduce CB when compared with the other products ($P < 0.05$). In conclusion, the products had the ability to gradually reduce lower concentrations of CB compared with higher concentrations of CB and were more effective at 8 h than at 0 h or 4 h. Further research is needed to investigate the reduction potential of the products when exposure is for more than 8 h.

Key Words: antibiotic alternatives, *Bacillus*, *Clostridium*, poultry, probiotics

163 Effect of sodium bisulfate salt on mitigating the presence of an antibiotic resistant strain of *Salmonella* Enteritidis on whole chicken drumsticks. D. Dittoe*[1], J. Atchley[1], K. Feye[1], L. Meyer[1], C. Knueven[2], and S. Ricke[1], [1]*University of Arkansas, Fayetteville, AR,* [2]*Jones-Hamilton Co., Walbridge, OH.*

The presence of *Salmonella* spp. on poultry products is one of the leading causes of foodborne illness in the United States. Therefore, novel antimicrobial substances are being explored as potential interventions in poultry processing facilities. Thus, the objective of the current study was to evaluate the efficacy of varying concentrations of sodium bisulfate salt, SBS, alone (1, 2, and 3%) or in combination with peracetic acid, PAA, (200 ppm) in 15 s whole part dips on mitigating the presence of inoculated nalidixic acid resistant *S.* Enteritidis on whole chicken drumsticks. Per replication (4), 24 drumsticks (8 treatments; 3 d) were inoculated in a 400 mL solution of nalidixic resistant (NA) *S.* Enteritidis (10^7 cfu/mL) and allowed to adhere for 60–90 min at 4°C for a final concentration of 10^4 cfu/g of NA resistant *S.* Enteritidis. The experimental treatments included: a no dip negative control (1); a 15 s dip in 300 mL of tap water (TW), positive control (2); TW+1% SBS (3); TW+2% SBS (4); TW+3% SBS (5); TW+1% SBS+PAA (6); TW+2% SBS+PAA (7); and TW+3% SBS+PAA (8). After treatment, drumsticks were stored under 4°C refrigeration until microbial sampling occurred. On d 0, l and 3, drumsticks were rinsed in 150 mL of neutralizing buffer for 1 min, then 100 µL of rinsate was serially diluted, spread plated on XLT 4+NA (20 µg/mL) to enumerate NA resistant *S.* Enteritidis. XLT4 plates were incubated under aerobic conditions at 37°C for 24 h. Log-transformed counts were analyzed using a randomized complete block design with split plot over time. Means were separated using Fishers protected LSD when $P \leq 0.05$. In the current study, a treatment effect ($P < 0.0041$) was observed. The 15 s dip of TW+1% SBS+PAA (3.16 Log cfu/g) and TW+2% SBS+PAA (3.14 Log cfu/g) resulted in a significant lower concentration of NA resistant *S.* Enteritidis on drumsticks compared with those untreated (3.60 Log cfu/g), treated with TW alone (3.40 Log cfu/g), or with 1% SBS+TW (3.40 Log cfu/g). However, the mean Log cfu/g of S. Enteritidis on drumsticks did not differ between the 15 s dip of drumsticks in TW+1% SBS+PAA, TW+2% SBS+PAA, TW+2% SBS, TW+3% SBS, and TW+3%SBS+PAA (3.16, 3.14, 3.30, 3.24, and 3.22 Log cfu/g respectively). No differences were detected between mean Log cfu/g of NA resistant *S.* Enteritidis on d 0, 1, and 3 of refrigeration ($P > 0.05$). In conclusion, the application of 1% and 2% SBS in combination with 200 ppm of PAA is capable of mitigating the presence of an antibiotic resistant strain of *S.* Enteritidis and maintaining that reduction over a 3-d refrigeration period; thus, potentially increasing the safety of poultry products for consumer consumption.

Key Words: *Salmonella* Enteritidis, sodium bisulfate salt, antimicrobial, processing, chicken drumsticks

164 Bacitracin methylene disalicylate reduces multidrug resistant *Salmonella enterica* serovar Heidelberg in the cecum of commercial broiler chickens. G. Dewi*, D. V. T. Nair, C. Peichel, S. Manjankattil, J. Langlie, and A. K. Johny, *University of Minnesota, Saint Paul, MN.*

Antibiotics have been shown to increase the growth performance in poultry, one of the potential mechanisms could be their activity against pathogens. Bacitracin methylene disalicylate (BMD) is a polypeptide antibiotic approved for use in poultry to improve feed efficiency, and to prevent coccidiosis-causing protozoan, Eimeria, and necrotic enteritis-causing gram-positive bacteria, *Clostridium perfringens*. Broilers are also reservoir hosts to a major gram-negative food-borne pathogen, *Salmonella* Heidelberg (SH), that has emerged in poultry, with high potential to cause invasive infections in humans. Along with its complex outer membrane, the drug resistance mechanisms in SH may potentially help to reduce its susceptibility to antibiotics such as BMD. Our objective of the current study was to determine the efficacy of BMD against multidrug resistant SH in broiler chickens. A total of 4 challenge studies were conducted. Two studies each were carried out using 2-week-old chicks (10 birds/group; 30 birds/study) and 4-week-old chickens (3 birds/group; 9 birds/study). The treatments included a negative control (NC), *Salmonella* control (PC), and BMD (50 g/ton) supplemented group (AB). Birds in the AB groups received feed supplemented with BMD from d 1 throughout the study whereas, birds in the NC and PC groups received standard commercial broiler diets. All birds except those in the NC groups were inoculated on wk 2 (2-week-old chicks) or on wk 4 (4-week-old chickens) with 5.2- and 3.8 $\log_{10}$ cfu SH (2014 Tennessee correctional facility outbreak strain), respectively, by crop gavage. All birds were euthanized after 7 d post-inoculation, and the surviving SH populations in the cecum were enumerated. Power analysis was conducted to determine the number of birds in both challenge models to determine meaningful SH reductions. The samples from which no bacteria were recovered after spread plating, but positive after enrichment, were assumed a value of 0.95 for analysis. The data were analyzed as a mixed effects model using lmerTest package of R. Significance was set at $P < 0.05$. In all studies, BMD supplementation significantly reduced MDR SH in the cecum compared with the *Salmonella* controls. An average SH reduction of 4.5- and 3.0- $\log_{10}$ cfu/g of cecal contents ($P < 0.05$) was observed in the 3- and 5-week-old broiler chickens (7 d after challenge), respectively. The results showed that BMD was highly detrimental on the MDR SH strain under study in the cecum of chickens, indicating its potential to contribute to the preharvest safety of broilers.

Key Words: bacitracin methylene disalicylate, *Salmonella* Heidelberg, broiler, antimicrobial, preharvest safety

165 Effects of functional ice on food safety, shelf-life, and quality of raw poultry thigh meat during transportation and storage. J. Kataria*, A. Smith, M. Johnson, E. Ariza, L. Garner, and A. Morey, *Auburn University, Auburn, AL.*

Food safety and microbiological spoilage are 2 major concerns during transportation and storage (TS) of raw poultry meat. Antimicrobial interventions applied during slaughter and debone operation may not have any residual antimicrobial efficacy and may fail to control pathogen and spoilage microorganism growth during TS. There is a need to develop a "sustained release mechanism" which can exert antimicrobial pressure during TS to improve food safety and shelf-life. The research was conducted to investigate the effects of "functional ice" (FICE) (Patent pending), an innovation over the commonly utilized ice, on food safety shelf-life and quality of raw poultry thigh meat during TS. FICE was prepared using an aqueous solutions of sodium tripolyphosphate

(STPP) (2.5% and 5% wt/vol) and sodium lactate-sodium diacetate (L+D) (1% and 2.5% vol/vol). Ice made from tap water acted as a control. For the food safety study, Nalidixic acid resistant (35µg/mL) *Salmonella* Typhimurium suspension was inoculated (100 uL) on fresh, raw boneless, skinless thigh meat pieces, allowed 1h attachment time (final inoculum 10^6 cfu/sample), placed in FICE treatments, stored in walk-in coolers (4C) to simulate transportation and sampled at 0, 12, 24, 36 and 48h (3 samples/sampling time × 5 trials). Thigh rinsates (30 mL/thigh) were spread-plated (0.1 mL) on XLT4 agar + nalidixic acid (35µg/mL), incubated at 37C for 24 h and the viable *Salmonella* colonies were reported as log cfu/mL of rinsate. For the shelf-life study, thigh meat was stored in FICE treatments (STPP 5% and L+D 2.5%) and tap water ice for 48 h. FICE treated thighs were packed in styrofoam trays with an absorbent pad (4/ tray), saran wrapped, stored at 4C and sampled on alternate days for 12 d. Microbiological analysis (10 samples/treatment × 7 sampling days × 3 trials) was conducted to determine the changes in aerobic plate counts, psychrotrophic plate counts, and lactic acid bacteria counts. Quality parameters, color, (L*, a*, and b*), and texture was determined at each sampling day. Significant differences among treatments were determined using ANOVA with Tukey's LSD at $P < 0.05$. *Salmonella* levels reduced ($P < 0.05$) by > 1 log within 12h of treatment with STPP 2.5 and 5% FICE compared with control treatment during the 48h refrigerated storage. Suppression of aerobic plate counts and lactic acid bacteria counts in the STPP 5% and L+D 2.5% FICE compare with control indicates the extended effects of FICE during storage. The results demonstrated that FICE has the potential to improve the food safety and shelf-life while maintaining yields during raw poultry storage and transportation.

Key Words: functional ice, *Salmonella*, shelf life, poultry

166 Development of an automated food safety intervention on poultry frames using hydrogen peroxide and ultraviolet light.
A. M. Jones-Ibarra*[1], J. Byrd[2], C. Coufal[1], and C. Alvarado[1], *[1]Texas A&M University, Bryan, TX, [2]USDA-ARS, College Station, TX.*

Poultry frames are used as rendered products for in pet food.The objective of this study was to examine the efficacy of a hydrogen peroxide (H_2O_2) and UV light combination treatment using a prototype machine to reduce a *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (ST) cocktail on poultry frames and to determine the effects on total aerobic plate counts (APC). Fresh chicken frames were spot inoculated with the cocktail to achieve a recovery level of 5 log cfu/g on each frame. Treatments included a negative control (not inoculated, no treatment), positive control (inoculated, no treatment), a manual spray of deionized water only (DI H_2O), and treatments using the H_2O_2/UV sanitization machine which included DI H_2O, 5.0% or 7.0% H_2O_2. Each treatment was replicated on 3 frames and 3 separate trials were conducted for this experiment. Machine experimental frames were treated with each solution using sprayers in the machine followed by UV light and repeated (total of 2 applications of treatment) followed by a final manual H_2O_2 rinse. After treatment, each frame was rinsed with buffered peptone water (BPW) and analyzed using serial dilutions to determine the reduction in *Salmonella* and APC on the frames. Recovered *Salmonella* serovars were transformed to $\log_{10}$cfu/frame before analysis of data. These data were analyzed using 2-way ANOVA and means were then separated using Student's *t*-test ($\alpha = 0.05$). Both concentrations of H_2O_2+ UV had the highest reductions in *Salmonella* ($P \leq 0.001$) with a 1.08 Log_{10} cfu/frame (5.0% H_2O_2) and 0.99 Log_{10} cfu/frame (7.0% H_2O_2) reduction compared with the positive control. DI H_2O+ UV also resulted in a significant ($P \leq 0.001$) reduction of *Salmonella* by 0.66 Log_{10} cfu/frame when compared with the positive control. The 5.0% H_2O_2 and 7.0% H_2O_2 treatments showed a significant ($P < 0.05$) reduction of 0.42 and 0.33 Log_{10} cfu/frame, respectively, compared with the DI H_2O+ UV. Both concentrations of H_2O_2 + UV had significant reductions ($P \leq 0.05$) of APC resulting in 0.80 Log_{10} cfu/frame for 5.0% $H_2O_2$and 1.50 Log_{10} cfu/frame for 7.0% H_2O_2 compared with the positive control, while DI H_2O+ UV did not result in a significant ($P \geq 0.05$) reduction when compared with the positive control. These findings indicate that all treatments in this study can reduce SE/ST on poultry frames while the 5.0% H_2O_2 and 7.0% H_2O_2 + UV light treatments can reduce both SE/ST and APC on poultry frames.

Key Words: *Salmonella*, poultry frame, pet food, rendering, mechanically separated chicken

Student Competition: Metabolism and Nutrition, Vitamins and Minerals II

167 Effect of choline chloride on the stability of cholecalciferol (vitamin D_3) sources used in animal nutrition evaluated by two different assays. K. Gardner*, H. Leyva-Jimenez, and C. Bailey, *Texas A&M University System, College Station, TX.*

Vitamins are coenzymes of metabolic reactions and therefore, even when required in small quantities, are considered dietary essential. Vitamins can be inactivated due to chemical interaction with other nutrients within the feed premix, ultimately, reducing their biopotency. To investigate the effect of choline chloride on the stability of vitamin D_3, a completely randomized design utilizing 2 concentrated stabilized commercially available D_3 products: a spray-dried fine powdered (P) source and a beadlet (B) type source were compared with 2 different methodologies. A basal premix was created to contain a carrier (diatomaceous earth), a commercial trace mineral premix and choline chloride-60% (Balchem Corp.) supplemented at 3 increasing concentrations (6%, 12% and 24%). Both D_3 sources were supplemented to the basal premix to yield 4,000 IU D_3 /g of premix. For the first assay (A1), premix treatments were stored in a closed outdoor metal storage room at ambient summer temperature. Temperature and relative humidity were monitored throughout a 9 wk period. Samples were taken at 0, 3, 6, and 9 wk. For the second assay (A2), leftover material from previous assay was remixed and 50 g samples were placed in plastic weigh bowls inside a cabinet-type egg incubator at constant temperature (40°C) and relative humidity (80%) for 14 d. Samples were taken at 0, 7 and 14 d. Collected samples from both assays were sent to a commercial laboratory to analyze total D_3 concentration. Data were subjected to ANOVA for a 2 (D_3 source) × 3 (choline chloride-60% level) factorial arrangement of treatments using the least squares function of JMP 13.0. Tukey's Test was used to identify significant ($P < 0.05$) differences when appropriate. Results for A1 showed no main effect or interactions ($P > 0.05$) for choline chloride on D_3 concentration. However, 24% choline chloride reported the lowest concentration for both sources after 9 wk storage. Additionally, a decreasing trend ($P < 0.05$) in D_3 concentration was observed at the 6–9 wk period overall for all treatments. The beadlet source showed better stability ($P < 0.05$) than the powdered source after 9 wk storage. For A2, increasing choline chloride concentrations in the premix decreased D_3 concentration linearly ($P < 0.05$) as early as 7 d after storage for both D_3 sources. In conclusion, vitamin D_3 concentration was negatively affected by choline chloride; this effect was more evident when D_3 sources were subjected to constant temperature and relative humidity inside the egg incubator. A2 resulted in a higher stability challenge for both D_3 sources and appeared to be more sensitive to D_3 concentration changes than A1.

Key Words: vitamin stability, choline chloride, cholecalciferol, vitamin D_3, animal nutrition

168 Effect of increased dietary choline chloride concentrations on growth performance and carcass characteristics of broiler chickens reared to 32 days of age. L. Spencer*[1], A. Calderon[1], O. Tejeda[1], K. Estes[2], B. Barton[2], T. Powell[2], J. Starkey[1], and C. Starkey[1], *[1]Auburn University, Auburn, AL, [2]Balchem Inc., New Hampton, NY.*

The last published study cited by the most recent *Nutrient Recommendations for Poultry* (NRC) investigating broiler chicken dietary choline concentrations that included the entire growth period was published in 1961. Thus, our objective was to determine the effect of additional dietary choline chloride supplementation on modern broiler growth performance and carcass characteristics of broilers reared to 32 d of age. Standard corn and soybean meal-based diets were formulated to contain an additional 0, 400, 800, 1,200, 1,600, or 2,000 mg of choline chloride per kg of feed above that provided by the ingredients in the basal diet. All nutrients were included in the diets at or above the breeder recommendations. Of particular importance, methionine concentrations were not limiting. Initial choline concentrations in the starter and grower basal diets were 1,183 and 1,171 mg per kg, respectively. The 6 experimental diets were provided in 2 phases: starter from d 0 to 15 and grower from d 16 to 32. On d 0, as-hatched, commercial Aviagen Yield Plus × Ross 708 broiler chicks (n = 2,160; 30 birds per pen) were randomly allotted and placed in 2.3 m^2 pens bedded with new wood shavings and equipped gravity-flow feeders and nipple drinker water lines. All birds and feeders were weighed at the end of each dietary phase to determine mortality-adjusted BW gain (BWG), feed intake (FI) and feed conversion ratio (FCR). On d 32, birds from the 6 different treatments were processed (n = 1,026) to determine carcass characteristics. Incidence and severity of both Wooden Breast and White Striping were evaluated following a 24-h static water chill utilizing a 0–3 scoring system. Continuous and proportional variables were analyzed using the MIXED and GLIMMIX procedures of SAS (v9.4), respectively. Means were considered significantly different when $P \leq 0.05$. No differences were observed among treatments for BWG, FI, or FCR ($P \geq 0.6153$). Carcass part weights were similar among dietary treatment (breast, tender, wing, drum, thigh, and abdominal fat pad; $P \geq 0.0698$). Broilers consuming diets with 800, 1,200, 1,600, and 2,000 mg per kg additional choline chloride had lower drum weights as a proportion of chilled carcass weight as compared with those receiving 0 additional choline ($P = 0.0219$). Increasing additions of choline chloride did not impact the incidence or severity of Wooden Breast or White Striping ($P \geq 0.4117$). Our results indicate that feeding additional dietary choline chloride above that provided in the basal diet may result in changes in processing characteristics of commercial broilers reared to 32 d of age.

Key Words: choline chloride, broiler, carcass, growth performance, myopathy

169 Effect of supplemental dietary choline chloride on growth performance and carcass characteristics of broiler chickens reared to 66 days of age. A. Calderon*[1], L. Spencer[1], O. Tejeda[1], K. Estes[2], B. Barton[2], T. Powell[2], C. Starkey[1], and J. Starkey[1], *[1]Auburn University, Auburn, AL, [2]Balchem Inc., New Hampton, NY.*

The last published study cited by the most recent *Nutrient Recommendations for Poultry* (NRC) investigating broiler chicken dietary choline concentrations that included the entire growth period was published in 1961. Therefore, our objective was to determine the effect of increasing choline chloride concentrations on modern broiler growth performance and carcass characteristics. The 6 experimental dietary treatments consisted of corn and soybean meal-based diets formulated to contain an additional 0, 400, 800, 1,200, 1,600, or 2,000 mg of choline chloride per kg of feed above that provided by the ingredients in the basal diet. All nutrients were included in the diets at or above the breeder recommendations. Of particular importance, methionine concentrations were not limiting. Initial choline concentrations in the starter, grower, and finisher basal diets were 1,183, 1,171 and 903 mg per kg, respectively. Diets were provided in 4 phases: starter from d 0 to 15, grower 1 from

d 16 to 28, grower 2 from d 29 to 49, and finisher from d 50 to 66. On d 0, as-hatched, commercial Aviagen Yield Plus × Ross 708 broiler chicks (n = 2,160) were weighed, randomly allotted to each treatment, and placed in floor pens bedded with new wood shavings and equipped with gravity-flow feeders and nipple drinker water lines. The breeder recommendations for both bird rearing temperature and lighting program were used. Birds and feed were weighed at the end of each phase to determine mortality-adjusted body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR). On d 66, birds from the 6 different treatments were processed (n = 907) and deboned (n = 611) to determine carcass characteristics. Incidence and severity of both Wooden Breast and White Striping were evaluated following a 24-h static water chill utilizing a 0–3 scoring system. Continuous and proportional variables were analyzed using the MIXED and GLIMMIX procedures of SAS (v9.4), respectively. Means were considered significantly different when $P \leq 0.05$. Broiler growth performance and mortality as well as Wooden Breast and White Striping scores were similar among treatments ($P \geq 0.2342$). Adding 1,600 mg choline chloride per kg of feed to the basal corn-soybean meal diet resulted in highest breast ($P = 0.0094$), wing ($P = 0.0083$) and thigh ($P = 0.0351$) weights as a proportion of chilled carcass weight. Our results indicate that feeding additional dietary choline chloride above that provided in the basal diet may result in changes in processing characteristics of commercial broilers reared to 66 d of age.

Key Words: choline chloride, broiler, carcass, myopathy, growth performance

170 Effects of light intensity and dual light choice treatments on plasma corticosterone and brainstem serotonergic activity of male broilers. S. Kang*[1], K. Christensen[2], D. Aldridge[1], and W. Kuenzel[1], *[1]University of Arkansas, Fayetteville, AR, [2]Tyson Foods Inc., Springdale, AR.*

The objective of this study was to investigate effects of light intensity and dual light intensity choice on plasma corticosterone (CORT), and tryptophan hydroxylase 2 (TPH2, the rate-limiting enzyme of serotonin biosynthesis) gene expression in the brainstem of male broilers. Day old broilers were housed in 2 commercial houses, and placed in 20 pens (10 pens/house, 11 birds/pen, 8 pens/trt). All the treatment groups were provided with 23h light (L) / 1h dark (D) and 40 lx (lx) light intensity during the first week and then 18L:6D (10 lx) from d 7 to 14. Blood and brain were sampled at 14 d of age (10 lx) before the onset of light treatments. On d 15, 4 treatments (2, 10, 20, and 100 lx), and one choice treatment (2/20 lx) were initiated. Samples were collected on d 15, 16, 17, 30 and 41. Plasma CORT levels were determined by radioimmunoassay (RIA). TPH2 mRNA expression in dissected tissue of the dorsal raphe nucleus (DRN) and caudal raphe nucleus (CRN) of brainstem was determined by qPCR. Differences among treatment groups were analyzed using one-way ANOVA followed by mean separation using the Tukey's HSD test (significance level of $P < 0.05$; JMP 11). RIA results showed that CORT levels of 100 lx and choice birds were significantly lower compared with birds exposed to 2, 10, and 20 lx on d 16 and 17 ($P < 0.05$). CORT levels of d 30 and 41 birds were not significantly different among treatment groups, suggesting the attenuating short-term effect of bright light (100 lx) and choice (2/20 lx) treatments on plasma CORT, and the extenuation of bright light and choice treatment effects on plasm CORT in long-term treatment birds. TPH2 expression in the DRN was significantly lower in the choice birds compared with other treatment birds on d 15 and 17, and was higher in the 2 lx treatment group compared with other treatments on d 16 and 17. On d 30 and 41, choice birds showed lower TPH2 expression levels compared with the 2 and 10 lx groups. TPH2 expression patterns in the CRN were different to those in DRN, showing that opposite TPH2 expression pattern on d 17 among treatments, and significant lower expression in DRN and CRN of 100 lx and choice birds on d 30 and 41, respectively. Taken together, temporal and spatial regulation of TPH2 expression and CORT plasma levels changes among treatment birds suggest that compensatory mechanism of TPH2 expression may occur in the least stressed choice treatment birds. Results suggest a beneficial effect of the light intensity choice treatment on broiler welfare. This research was supported by a Tyson Research Grant.

Key Words: light intensity, corticosterone, choice preference, serotonin, TPH2

171 Effects of infrared beak treatment on the behavior, welfare, and mortality of egg-strain pullets and hens to 60 weeks of age. S. Struthers* and K. Schwean-Lardner, *University of Saskatchewan, Saskatoon, SK, Canada.*

The objective of this study was to determine the impact of infrared beak treatment (IRBT) on behavior and welfare indicators. We used Lohmann LSL-Lite (LW) and Lohmann Brown (LB) pullets and hens, in a 4x2 factorial arrangement of IRBT and bird strain, in a randomized complete block design. IRBT settings (day of hatch) were adjusted to create 4 beak shapes: shovel (SHV) (top beak much shorter than bottom), step (STP) (intermediate differentiation), standard (STAN) (small differentiation) and sham treated control (C). Pullets (n = 640) were floor reared (2 pens per IRBT × strain) from 0 to 18 wk, and behavior was video recorded using 2 reps per IRBT × strain for one 24-h period at 5, 9, 13 and 17 wk. At 18 wk, hens (n = 576) were housed in cages (6 reps per IRBT × strain, 12 birds per rep) to 60 wk. Hen behavior (3 reps per IRBT × strain) was video recorded (24-h) at 23 and 39 wk, and analyzed using scan sampling at 15 min intervals. Hens were scored for feather cover and comb damage at 18, 42 and 60 wk. Mortality was recorded daily. The effect of IRBT, strain, and interactions were analyzed using Proc Mixed (SAS 9.4) with Tukey's range test to separate means. Differences were significant when $P \leq 0.05$. Beak treatment impacts on behavior were minor. At 5 wk, only the percent of time spent in exploratory behavior differed, with C birds spending more time than STP and STAN birds. At 23 wk, SHV and STP hens preened more than C hens. There was no IRBT effect on behavior at 9, 13 and 39 wk. LB birds were more active (10.6 vs. 7.8%) and rested less (68.9 vs. 71.3%) than LW birds at 5 wk. LB birds were more active than LW (11.7 vs. 10.3%) at 9 wk. LW birds preened more than LB birds (6.8 vs. 6.5%) at 13 wk. At 23 wk, LB hens were more active but rested and preened less than LW hens. At 39 wk, LW hens engaged in more nutritive, resting and preening behaviors than LB hens. An interaction between IRBT and strain was noted for time spent walking at 17 wk, with C LW birds walking more than SHV LB, C LB, and STP LW birds. IRBT had an effect on feather cover and comb damage scores with C hens scoring poorest. IRBT or strain did not affect pullet or hen mortality. LW birds had higher infectious mortality than LB birds (2.2 vs. 0.0%). An interaction between IRBT and strain was seen for hen mortality due to cannibalism, as C LB hens had higher mortality than treated hens of both strains (19.4 vs. 0.0%), and LB hens were removed from the trial at 42 wk. The results indicate that as birds reach sexual maturity, the minor effects on behavior noted with IRBT are no longer apparent, and that IRBT is effective at reducing feather and comb damage, and mortality from cannibalism.

Key Words: Lohmann, cannibalism, feather cover, comb damage

172 Ventilation shutdown in conjunction with CO_2 results in a quicker depopulation method with reduced stress. D. Hodgson*[1], K. Anderson[1], K. Eberle-Krish[1], M. Martin[2], S. Shah[1], R. Malheiros[1], and K. Livingston[1], *[1]North Carolina State University, Raleigh, NC, [2]North Carolina Department of Agriculture, Raleigh, NC.*

The need for ethical depopulation during emergency conditions for large layer flocks has risen due to the influx of new diseases. Welfare of the animals and speed of containment of the outbreak are of the upmost concern. The ideal standard for depopulation is for the animal to quickly enter insensible stage limiting the amount of stress. We evaluated the stress response of laying hens subjected to 4 different depopulation methods: ventilation shutdown (VSD), ventilation shutdown + heat (VSDH), ventilation shutdown + CO_2 (VSDCO), and ventilation shutdown + heat + CO_2 (VSDHCO). In phase 1, blood was collected before treatment and immediately after death. In phase 2, blood was collected before treatment and evenly spaced intervals as determined by phase 1. Corticosterone (CORT) was used as an indicator of the stress response of the birds during depopulation. CORT was extracted using the Extrelut method, followed by Caymen Chemical Corticosteron ELISA and evaluated. The statistics were run by 2-way ANOVA with repeated measures using SAS 9.4. Time of death was compared with CORT levels. EEG data was used to determine point of the insensible

stage and time of death. In phase 1, VSD had the least amount of CORT (1.14 ng/ml) while VSDHCO had the greatest CORT level at 2.92 ng/ml ($P < 0.05$). VSDH and VSDCO had intermediate levels of CORT at 2.12 and 2.08 ng/ml. However, it took VSD birds significantly longer to reach the time of death than the other methods ($P < 0.05$). Phase 2 shows that birds subjected to VSD initially have a spike in CORT, which over time is exhausted. While other treatments with shorter intervals to reach time of death, increased CORT. In conclusion, although layers subjected to VSD had lower CORT level at time of death, this is most likely due to adrenal exhaustion rather than reduced stress.

Key Words: depopulation, corticosterone, ventilation shutdown

173 A cross-sectional study on feather cover damage in laying hens in non-cage housing systems. N. van Staaveren*, C. Decina, C. Baes, T. Widowski, O. Berke, and A. Harlander-Matauschek, *University of Guelph, Guelph, ON, Canada.*

Non-cage housing systems are increasingly being used worldwide in efforts to improve laying hen welfare. However, feather damage (FD) due to feather pecking remains a welfare concern in these systems where birds are housed in large groups. The aim of this study was to identify bird, housing and management associations with FD in laying hens in non-cage housing systems in light of Canada's commitment to phasing out conventional cages. A questionnaire encompassing housing and management practices, was developed and distributed to 122 laying farms nation-wide in autumn of 2017 (response rate of 52.5%), providing information on a subset of 39 flocks housed in non-cage systems. Prevalence of FD was visually assessed by farmers using a simple FD scoring system (0: fully feathered, 2: poorly feathered with a naked area larger than a 2-dollar coin). Fifty birds proportionately selected from the litter, slatted area and tiers were assessed and the percentage of birds affected by FD (score >0) was calculated. Variables that met the univariable selection criteria ($P < 0.25$) or were considered biologically relevant, were used in preliminary multivariable linear regression modeling in R. Litter provision, frequency of manure removal/manure belt operation, bird age, enrichment provision, matching of rearing and laying environment by providing litter substrate, and provision of a dawn/dusk period were included in the final model and accounted for 64% of the variation in FD between farms. Age (+0.9% ± 0.29) and housing of birds in all wire/slatted barns compared with all litter barns (+37.6% ± 13.1) were associated with higher prevalence of FD. Additionally, on farms where manure was removed only at the end of the flock, FD prevalence tended to be higher (+20.1% ± 10.70). Provision of enrichment was also associated with higher FD (+19.1% ± 8.04), which is likely an indication that enrichment was provided after FD was observed as a control measure. These results highlight the importance of providing litter substrate and proper litter management (e.g., via prevention of manure accumulation on litter substrate) to reduce FD in non-cage housing systems in Canada. However, these practices need further examination in longitudinal and/or intervention studies on commercial farms to verify their use as management strategies to reduce FD in hens housed in non-cage systems.

Key Words: laying hen, feather damage, aviary, floor system, litter

174 Effects of functional sensory molecules on the stress level and the growth performance of broilers under two different stocking density. J.-F. Gabarrou*[1], A. Wagner-Wells[2], and P. Castañeda[3], *[1]Phodé Sciences, TERSAC, France, [2]Probiotec International, Sainte Hyacinthe, Canada, [3]University of La Molina, La Molina, Peru.*

High stocking density reduces broiler performance by negatively impacting feed intake and growth. Veo, a Functional Sensory Molecules blend (FSM) from sweet orange essential oil (rich in D-limonene, PHODE, Albi, France) was designed to mitigate animal stress perception. The objective of this study was to evaluate the FSM effect on broilers subjected to density stress. One-day-old Cobb chicks were dispatched into 8 pens × 3 treatments (24 pens). Treatment 1 (T1) was the negative control with low stocking density (10/m^2) and treatment 2 (T2) served as the positive control with a high stocking density (12/m^2). T1 and T2 did not receive FSM. Treatment 3 (T3) received FSM in the feed (250g/t) from day 1 to day 42 in a high stocking density environment. At day 42, 2 broilers per pen underwent an immobility test (Gordon & Galup, 1974) to determine stress level; where, the frequency of birds that took more than 20 seconds to stand up were categorized as stressed. Stress level was also determined based on cortisol and the ratio between blood heterophyles vs. total lymphocytes (H/L) was measured with blood samples from one broiler per pen at day 42. Feed intake (FI), live BW and feed conversion ratio (FCR) were determined at day 42. Statistical analyses were performed on all parameters with a one-way ANOVA with treatment as fixed factor (SPSS v25), and a Chi^2 test was used to analyze the immobility test. Significance was considered at $P < 0.05$. The frequency of animals that took more than 20 seconds to stand following the immobility test was higher ($P < 0.001$) for T2 (62.5%) than T1 (18.8%) and T3 (17.3%). Blood cortisol was also elevated ($P = 0.05$) for T2 (21.1±2.9ng/ml) birds compared to T1 (17.3±2.1ng/ml) and T3 (17.3±1.9ng/ml). However, there was no effect ($P = 0.13$; T1: 0.96±0.07; T2: 0.99±0.05; T3: 0.92±0.09) of treatment on H/L ratio. All of the examined growth parameters were impacted by treatment; where FI was reduced ($P < 0.001$) in T2 birds (5290±17g) compared to T1 (5537±29g) and T3 (5353±22g). Similarly, BW of T2 birds was less ($P < 0.001$; T2: 3115±22g) than T1 (3308±12g) and T3 (3190±35g). As a result, T2 birds (1.72±0.01) demonstrated elevated FCR in comparison to T1 (1.70±0.01) and T3 (1.70±0.02). The results of the immobility test as a measure of behavioral stress and the blood cortisol levels as a measure of physiological stress demonstrated that the stocking density model was a stressor. Additionally, these results indicate the benefits of FSM to mitigate environmental stress. Furthermore, these benefits were demonstrated by the beneficial impact on the growth performance parameters. Additional research is warranted to examine the effects of FSM on other stressors.

Key Words: stocking density, functional sensory molecule, stress, broiler

175 Using on-animal sensors to assess northern fowl mite effects on poultry behavior and welfare. A. Murillo*[1], A. Abdoli[1], R. Blatchford[2], and A. Gerry[1], *[1]University of California Riverside, Riverside, CA, [2]University of California Davis, Davis, CA.*

The northern fowl mite (NFM), *Ornithonyssus sylviarum,* is the most common and damaging ectoparasite of poultry in the United States. It is an obligate blood-feeding mite that causes direct damage to birds and decreases economic output. Severe infestations and economic damage occur primarily in layers which are raised for long periods of time. Despite this impact, little research has focused on measuring the welfare of hens with NFM infestations. Linking mite infestations to welfare metrics is a knowledge gap in understanding and optimizing high standards of animal welfare in poultry production. In this study, the physical condition and behavior of 4 cage-free flocks of 12 Hyline brown layers were tracked before, during, and after mite infestation. A modified version of the Welfare Quality Assessment for poultry (WQA) was used to assess physical condition, while on-hen sensors, which

measure magnitude of force along 3-axes, were used to identify and quantify pecking/feeding, grooming, and dustbathing. At Week 1, WQA measures were recorded for each bird (no mites present). Accelerometers were then placed on each bird for one week. WQA and sensor readings were repeated at Week 4 (low mite levels present) and Week 7 (high mite levels). At Week 9, all birds were individually treated with an acaricide (RAVAP) to remove all mites. WQA and sensor readings were recorded at Week 12 (no mites). Mites were then introduced to each bird in 2 of 4 flocks at Week 14, to allow for a head-to-head comparison between mite-infested and uninfested birds; the final WQA and sensor readings were recorded at Week 19. Mite populations were scored for each bird weekly from Week 4 – Week 19. Average mite scores (0–7) for all flocks were: Week 1 0.0 ± 0.0; Week 4: 2.10 ± 0.22; Week 7: 4.83 ± 0.14; Week 12: 0.0 ± 0.0. WQA measures were analyzed using Fisher's exact test. Preliminary results indicate differences among comb wounds ($P < 0.0001$) and skin lesions ($P < 0.001$) at Week 7 when mite populations were high on chickens. Sensor output was analyzed using an algorithm that identifies instances of pecking, grooming, and dustbathing. Week 1 was used as a baseline for all behaviors, and average counts for all flocks at Week 1 was: Pecking: 2896 ± 1238, Grooming: 512 ± 40, Dustbathing: 24 ± 4. The biggest differences in weekly counts of behaviors were observed between Week 1 and Week 4 (low mites) for all behaviors (Pecking: +111%, Grooming: +44%, Dustbathing +37%). Variation of WQA and behaviors among individuals is high, though preliminary results indicate trends that relate to mite populations on hens. To our knowledge this is the first study that relates WQA and sensor behavior readings to ectoparasite burden over time.

Key Words: laying hen, ectoparasite, mite, behavior, welfare

176 Effects of microbiome on behavior and well-being. R. Dennis* and A. Patt, *University of Maryland, College Park, MD.*

The global modern poultry industry is facing numerous changes to feeding and supplementation regulations and restrictions. Although these differ greatly across countries and companies, the fast-paced changes in poultry feed supplements is ubiquitous, especially in regards to antibiotics, pro- and pre-biotics. Unfortunately, the majority of the research into these supplements has focused on production parameters with far less research into the welfare implications of these supplements. Still fewer studies investigate the impacts of the use of these products in young, developing birds. In the present study, 1 d old broiler chicks were given water treated with an antibiotic cocktail, probiotic cocktail, or no supplement (control). Treatment water was provided ad libitum for the first week of life and changed daily. Following the treatment week, all birds received untreated water for the remaining 4 weeks of the study. At wk 5 of age, tonic immobility (TI) durations were measured in 1 bird per pen, and flight distance from a novel human were measured in all pens (n = 12). Body and organ weights were taken as well as neural tissue samples for monoamine analysis. Probiotic treated birds had reduced TI durations compared with both control and antibiotic treated birds ($P < 0.05$). However, flight distances were greater in antibiotic treated birds compared with both probiotic and control birds ($P < 0.01$). No difference was observed in final body weight between the treatments ($P > 0.1$), however, antibiotic treated birds tended to have a greater heart weight than probiotic birds ($P = 0.072$) and greater spleen weight compared with control birds ($P = 0.057$). Antibiotic treated birds also expressed reduced levels of serotonin in the hypothalamic tissue compared with probiotic and control birds ($P < 0.05$). Sequencing analysis of the microbiome from fecal samples in this study indicates changes gut microflora of the 3 treatment populations over time. These populations are most different during early developmental stages. Our data shows the importance of investigating the effects of microbiome altering supplements on bird behavior and well-being. Additionally, we have shown that long lasting behavioral and physiological changes can be achieved by early ingestion of antibiotic and probiotic supplements. The long-lasting impacts of early antibiotic treatment inhibit positive welfare as indicated by increased fearfulness and reduced hypothalamic serotonin.

Key Words: broiler, microbiom, behavior

177 Health, production, and resource use by laying hens in an aviary: A risk assessment. A. Ali*[1], D. Campbell[2], D. Karcher[3], and J. Siegford[1], *[1]Michigan State University, East Lansing, MI, [2]Armidale, Armidale, NSW, Australia, [3]Purdue University, West Lafayette, IN.*

Different strains of commercial laying hens have been molded by varying selection pressures, impacting their production, health, and behavioral traits. Therefore, assumptions that all laying hen strains use the given resources within aviary systems similarly and maintain equal health and performance may be false. The influence of laying hen strain (Hy-Line Brown, Bovans Brown, DeKalb White, Hy-Line W36) on resource-use, egg production and health within an aviary system across the lay cycle was examined. Risks associated with differences in hens' resource use on egg production and health were calculated. Hens' distribution among different resources (litter areas, nests, wire floors, ledges, and perches) were recorded at different times of day (Light and Dark) for 3 consecutive days, per production stage (peak-lay: 28 wk, mid-lay: 54 wk and end-lay: 72 wk). Daily egg production (total, nest, and non-nest laid eggs) was recorded and 15% of hens/unit were randomly selected and assessed for keel bone damage, foot health, and plumage quality. Observations were conducted in 16 aviary units (4 units/strain, 144 hens/unit). Strain differences were assessed using GLMM with Tukey's Post hoc test applied to significant results. Production and health risks associated with hens' resource-use were assessed using multivariable regression modeling (α set at 0.05) in R 3.3.1. During the Light, brown hens used wire floors more than white, while higher numbers of white hens were observed using perches, litter, and ledges. During the Dark, brown hens were on lower tier wire floors more than white hens, while the latter occupied the upper tier at higher numbers. Brown hens laid more eggs outside nests, showed lower incidences of keel fractures and deviations, and better plumage quality than white hens. White hens had higher odds of keel fractures (4.2) and deviations (2.1) than brown hens. Odds of keel fractures and deviations, respectively, were 3.7, 2.9, and 5.7, 5.3 times higher during mid- and end-lay than at peak-lay in all strains ($P \leq 0.05$). Using the upper tier for nighttime roosting increased odds of keel fractures 5.4 times. Using perches was associated with higher odds of keel deviations (Light: 2.6, Dark: 4.23) and lower odds of foot lesions (Dark: 0.73) and poor plumage quality (Dark: 0.63) in all strains across lay ($P \leq 0.05$). Finally, lower odds of non-nest laying were associated with white hens (0.76), while higher odds of non-nest egg laying resulted from the higher use of nests and litter areas (1.56, 1.25, respectively) in all strains across lay ($P \leq 0.05$). Thus, distinct strain differences in resource use in an aviary were associated with different risks to hens' production, health, and welfare

Key Words: laying hens, aviary, welfare, keel fracture, resource use

178 Preparing poultry producers to develop an effective biosecurity plan. A. Neu*[1], K. Lautenschlager[2], S. Voss[2], S. Noll[3], and D. Lauer[2], *[1]University of Minnesota, Willmar, MN, [2]Minnesota Board of Animal Health, Willmar, MN, [3]University of Minnesota, St. Paul, MN.*

To ensure successful foundational National Poultry Improvement Plan (NPIP) Biosecurity Audits, University of Minnesota Extension (UMN Ext.) created outreach materials for Minnesota poultry producers. After point source introductions of H5N2 highly pathogenic avian influenza (HPAI) virus in 2014–2015, analysis and epidemiology indicated most cases were introduced by farm-to-farm spread. This evidence demands increased system and on-farm biosecurity to prevent or reduce future introductions. The industry chose to include basic biosecurity principles into the NPIP as accepted minimum management practices. A 14-Point Biosecurity Principles document including, a biennial audit, was proposed and passed by delegates at the 2016 NPIP Biennial Conference. The Official State Agency (OSA) overseeing NPIP programs in Minnesota is the Minnesota Board of Animal Health (MBAH). Acknowledging the need for producer education on new biosecurity principles, MBAH invited UMN Ext. to provide outreach for poultry premises in Minnesota. To ensure successful NPIP Biosecurity Audits, the training initiative assisted producers in learning how to develop site-specific biosecurity plans that can be sustained after the audit is complete. Program design and distribution began in the last quarter of 2017. Official NPIP documents were consulted to provide practical explanations and examples relatable to producers. Three online platforms were used to deliver information: YouTube (video), Google Blogger (written blog) and Google Drive (accompanying resources, templates and tools) for each biosecurity principle. To optimize engagement, information for one principle was released each week for 14 weeks through multiple outlets including MN Ext., MBAH and Minnesota's commodity organizations. Six record keeping templates, 14 blog posts and 14 YouTube videos exploring aspects of the Biosecurity Principles were created and delivered, and continue to be managed. From September 18 – December 31, 2017, educational videos posted on YouTube and blog posts had 1,181 and 1,195 views, respectively. Record keeping templates were accessed 130 times and incorporated in 29% of the submitted audits. To date, all audited premises in Minnesota have successfully passed the audit although the OSA requested corrective actions from 92% of the first set of audits. Participation in this web-based education indicates Minnesota poultry producers desire better understanding of NPIP Biosecurity Principles as they prepare for their initial audit. Further development and distribution of outreach materials for the NPIP Biosecurity Audits will continue in 2018 to optimize effectiveness and ensure the success of remaining foundational NPIP Biosecurity Audits in Minnesota.

Key Words: biosecurity, audit, National Poultry Improvement Plan, outreach, module

179 Discussing poultry welfare and sorting the differences between animal welfare and animal rights. D. Bourassa*[1] and L. Jacobs[2], *[1]Auburn University, Auburn, AL, [2]Virginia Tech, Blacksburg, VA.*

In today's marketplace, consumers are increasingly concerned with the origin of the meat they buy. However, with increasing separation between consumers and first-hand knowledge of animal agriculture, information regarding how poultry is produced is extremely variable and sometimes misleading (e.g., cage free broilers). Furthermore, information can be based on agendas not backed by scientific data. When discussing agricultural animal welfare with consumers, emphasis should be placed on responsible care, avoidance of unnecessary suffering, and lack of cruelty. When considering potential policy or regulation, impartial recommendations should be made based on measureable outcomes from peer-reviewed scientific data. Consumers can experience information overload and experience difficulty in decision-making due to uncertainty about which products align with their priorities. Animal welfare and animal rights priorities can span a range from improving welfare based on measured outcomes, to changing how poultry are raised for human consumption, to ending the raising of all poultry for human use and consumption. Three examples of the advocacy range include the Animal Agriculture Alliance, Global Animal Partnership (GAP), and Farm Animal Rights Movement (FARM). Animal Agriculture Alliance is composed of agricultural industry members with experience in animal agriculture, such as National Turkey Federation, United Egg Producers, and the National Chicken Council. This organization engages in animal welfare discussion and has a goal of informing on the role of animal agriculture in providing a safe, abundant food supply. GAP has a stated purpose of improving farm animal welfare. This organization's leadership is comprised of members from organizations including Compassion in World Farming, Humane Society International, American Society for the Prevention of Cruelty to Animals, Whole Foods Market, and others. GAP provides a 5-step certification program detailing standards for the growing and slaughter of both broilers and turkeys as well as pullet and laying hen standards. FARM has the goal of ending animal use for food. This organization includes organizational representatives including A Well-Fed World and International Vegetarian Union. When consumers are looking for information about their food, they will find a large span of information which may be difficult to sort without background knowledge of procedures used during animal agriculture. Oftentimes decisions can be made based on erroneous information. It is crucial for extension agents and agriculture educators to discuss the differences between animal welfare and animal rights with our program participants and students, as well as the general public.

Key Words: animal welfare, animal rights, extension

180 Integration of student e-portfolio use into the Auburn University poultry science curriculum. J. Starkey*, C. Starkey, and A. Morey, *Auburn University, Auburn, AL.*

Student e-portfolios are a valuable pedagogical tool gaining popularity at many higher education institutions. An e-portfolio (electronic portfolio) is an organized digital collection of documents and media (called artifacts) that are contextualized with the student's reflection on professional goals. An e-portfolio demonstrates the student's improvement in skills and knowledge over the course of their education. A well-designed e-portfolio gives prospective employers a more complete sense of what the student's experiences have been and what they are capable of compared with the typical 1-page, paper resume they often receive from undergraduate-level applicants. As students go through the process of creating, building, and improving their e-portfolios, they gain experience and build their communication and critical thinking skills. To develop an effective e-portfolio students must: 1) connect concepts from different courses and make connections between coursework and extra-curricular activities, 2) organize, synthesize, and integrate ideas from various experiences, 3) thoughtfully articulate both classroom and

extra-curricular experiences, and 4) answer critical questions about their professional goals. Personal reflection is typically the most difficult portion of the development of an effective student e-portfolio. Yet, it is also the most important part. Generation of the personal reflections in artifacts results in improvement of the student's critical thinking skills. These skills are essential for students to be life-long learners capable of success in the diverse jobs available in the poultry industry. The goal for integration of student e-portfolio use in the Auburn University Poultry Science (PS) curriculum is to have at least 2/3 of our courses requiring student e-portfolio use through course assignments that are designed for the generation of e-portfolio artifacts. Students first encounter e-portfolios in their introductory PS courses during their sophomore and early junior years, where the major focus is on building the e-portfolio and learning how to generate artifacts that contain significant personal reflection. As students reach their late junior and senior years, they begin to advance in their critical thinking skills and expand their e-portfolios using their experiences both in and out of the classroom, including those gained during their mandatory poultry industry internships. Integration of student e-portfolios into the Auburn University PS curriculum is designed to not only improve student learning and critical thinking competency, but also to improve our student's ability to articulate their experiences and technical competency, especially during a job search.

Key Words: student e-portfolio, electronic portfolio, artifacts, critical thinking, reflection

181 Further evidence that corticotropin releasing hormone neurons in the nucleus of hippocampal commissure are involved in stress responses in birds. H. Kadhim*, S. Kang, and W. Kuenzel, *University of Arkansas, Fayetteville, AR.*

The stress response in birds is regulated by corticotropin releasing hormone (CRH) and arginine vasotocin (AVT) neurons in the paraventricular nucleus (PVN). Recently, another neural structure, the nucleus of the hippocampal commissure (NHpC), has been proposed to regulate stress. A stress study was therefore designed to examine more closely gene expression of CRH and AVT neurons and their receptors within the PVN and the NHpC. Chicks were subjected to one of the following food deprivation (FD) treatments when birds were 14 d of age: 0h, 1h, 2h, 3h, 4h, and 8h of FD. At each time point, brains were sampled, frozen, and cryosectioned. The NHpC and PVN were to micro-punched for determining their gene expression using RT-PCR. One-way ANOVA, followed by mean separation among the 6 treatment groups, was conducted for each gene using a significance level of $P < 0.05$. In FD groups, CRH expression in the NHpC was increased at 1h, peaked at 2h ($P < 0.001$), then declined rapidly. CRH mRNA in the PVN likewise significantly increased at 1h, however, continued to rise through 8h of FD. Data show that the NHpC is involved in the initial response to FD while the PVN sustains the continued response as the FD stress effects become more intense. Importantly, expression of CRH-R1 and -R2 in the NHpC compared with the PVN were significantly different. In the NHpC, gene expression of CRHR1 and R2 were downregulated at 1h ($P < 0.001$) followed by a steady rise with highest expression at 8h ($P < 0.001$) showing that they display a negative feedback, responsible for shutting down expression of CRH neurons within the NHpC. In contrast, CRH-R1 and -R2 expression in the PVN were increased at 1h, peaked at 3h ($P < 0.001$) and remained elevated through 8h of FD suggesting a positive feedback effect of CRH on its own receptors in the PVN. AVT mRNA in the NHpC was downregulated at all time points ($P < 0.001$). This could be attributed to the absence of AVT cell bodies in the NHpC. However, a major AVT receptor, V1aR, was downregulated in the NHpC at 1h of FD, but increased quickly and peaked at 8h ($P < 0.001$). In contrast, AVT expression in the PVN was upregulated after 1h, peaked at 4h ($P < 0.001$) and declined, but remained significantly higher than controls at the last sampling point, 8h, while V1aR expression, within the PVN, showed no significant difference at 1h and 2h; its expression steadily increased from 3h to 8h ($P < 0.001$). The study suggests that both NHpC and PVN are involved in the FD response to stress via activation of CRH neurons. The NHpC displays the first significant response while CRH neurons within the PVN sustain the continued elevation of CRH gene expression.

Key Words: food restriction, paraventricular nucleus (PVN), CRH receptors, septum

182 Characterization of gene expression in turkey sperm storage tubules from non-inseminated hens over the course of reproduction. K. Krasnec* and J. Long, *USDA Agricultural Research Service, Beltsville, MD.*

Sperm storage in the female reproductive tract is a known biological feature for a wide range of species. In the turkey, sperm residing in the hen's sperm storage tubules (SST) retain fertilizing ability for 10 weeks after a single insemination; however, the molecular and cellular mechanisms are largely undefined. A subset of data from a larger study involving non-inseminated, sham-inseminated, and sperm-inseminated hens was analyzed to evaluate SST gene expression over time in the non-inseminated hens. Reproductive tracts were recovered at the onset (d 1, n = 3), peak (d 30, n = 4), and end (d 90, n = 3) of reproduction. The region of the utero-vaginal junction (UVJ) containing SSTs was dissected and flash frozen. Laser capture microdissection of cryosections was used to isolate and remove SST cells from the surrounding vaginal epithelial tissue for RNA extraction. Approximately 25–30 million fragments of 150bp paired-end reads per sample were generated using RNASeq (Illumina NextSeq). Sequences were aligned to the turkey reference genome and annotations were used to identify gene transcripts. Differential gene expression was assessed using pairwise comparisons (d 1 vs d 30; d 1 vs d 90; d 30 vs d 90) to identify genes that were significantly (+/ 1.5-fold change; $P < 0.05$) up- or downregulated. Differentially expressed genes were broadly categorized as: SST microenvironment; immune function; vascularization; cell structure; cell migration/proliferation; and apoptosis. Genes involved with lipid synthesis, metabolism, and peroxidation (e.g., CAT, CH25H, LPL, PLCD1) were upregulated at peak reproduction compared with onset and end. The majority of genes associated with potassium and calcium binding or channel formation (e.g., CALCRL, FBLN2, KCNJ15, KCNK5, TMTC1) were upregulated at the onset of reproduction; however at peak reproduction, KCNMB1, TRPV4 and TRPC4 were upregulated. Cell migration and proliferation genes (EPHB6, FSTL1, POSTN, PRKX) were upregulated at onset and declined at peak through the end of reproduction, except for CTGF which was upregulated at peak and declined at the end. Genes involved in apoptosis (ELMO1, AEN) were only found to be upregulated at the end of reproduction. Results indicate that specific genes are associated with the molecular function of the SST during the reproductive lifespan of commercial turkey hens. These insights, paired with examination of gene expression from the SSTs of inseminated hens, may enable a greater understanding of SST function that would help improve in vitro semen storage methods and/or fertility.

Key Words: reproduction, sperm storage tubules, turkey, gene expression, RNASeq

183 Effect of *Rosmarinus officinalis* leaf powder on performance, thyroid hormones and blood cells in broiler chicks. M. Modaresi*, *Islamic Azad university, Isfahan (Khorasgan) Branch, Esfahan, Iran.*

The present study was carried out to evaluate the effects of different levels of rosemary powder on performance, blood cells and thyroid hormones in broiler chickens. In this experiment, 576 one-day-old broiler chicks (Ross 308) were used in a randomized complete design with 9 treatments, 4 replicates and 16 chicks per replicate. Experimental treatments consisted of diets containing 0.5 and 1% rosemary powder, which were fed to birds during a 42-d experimental period. Mean feed intake, daily weight, and feed conversion ratio was recorded periodically. Growth performance parameters (body weight gain, feed intake and feed conversion ratio) were determined biweekly. At d 20 and 42 blood samples were taken to measure thyroid hormones (TSH, T_4, and T_3) and white blood cells. The data were analyzed by SAS (2008) and Means were compared for significant ($P \leq 0.05$) differences by using the LSMEANS. The results showed that in the growth and final stage, the average feed intake of chicks fed rosemary powder was significantly higher than that of the control group ($P < 0.01$). In contrast, the highest daily gain in the final period (25–44) was allocated to the groups

receiving 1% rosemary powder ($P < 0.01$). At 20 d of age, the highest concentration of thyroid hormones was related to treatment 1% and the lowest was related to 0.5% rosemary powder. The heterophile and lymphocyte count was significantly different between experimental treatments ($P < 0.05$). As consumption of rosemary powder decreased levels of heterophile and increased lymphocyte levels. At d 42, the highest concentrations of thyroid hormones were related to the 1% treatment and the lowest was 0.5% of the rosemary powder. According to the results of this study, it seems that the use of rosemary powder in the diet of poultry improves the growth performance and feed intake, and increases the level of thyroid hormones and increases the immunity of blood.

Key Words: broiler, rosemary powder, heterophil, lymphocyte, thyroid hormone

184 Effect of storage time, time of fasting, blood fractions, and light intensity on the levels of cholesterol and triglycerides to broilers. R. Nunes*[1], L. Wachholz[1], C. Souza[1], J. Broch[1], C. Souza[1], J. Damasceno[1], C. Eyng[1], and G. Pesti[2], *[1]Universidade Estadual do Oeste do Paraná, Marechal Candido Rondon, Brazil, [2]University of Georgia, Athens, GA.*

The effects of storage time, time of fasting, blood fractions (plasma *vs* serum) and light intensity (5 vs 20 lx) on the levels of cholesterol and triglycerides were studied in broilers. 140 45 d old male broilers (average weight of 3407 ± 269 g) were used. Initially the birds were fasted for 1 h and then were fed for 30 min. After ingestion of feed blood was collected every 2 h during some 12 h fast. Blood was collected from the ulnar vein, using vacuum tubes with and without anticoagulant (sodium fluoride + EDTA). After the blood was collected it remained at rest for 15 min and was centrifuged at 2500 rpm for 10 min, the plasma and serum were separated into eppendorf tubes and stored at −20 C. The storage times were 0; 15; 30; 60 and 120 d. Analyses were performed utilizing a biochemical autoanalyzer (Flexor EL 200) with specific kits (Elitech) and calibrated with standard ELICAL. Data were analyzed using the GLM procedure of SAS. There were no observed interactions between the blood fractions, storage time, time of fasting, or light. Cholesterol was significantly higher ($P < 0.001$) in the serum (138.38 ± 14.3) when compared with the plasma (123.13 ± 14.4). Cholesterol was significantly higher ($P < 0,001$) when the birds were housed with 5 lm (131.74 ± 16.5) compared with 20 lm (128.07 ± 15.8). Triglyceride levels were lower ($P = 0.03$) at 9 h and 5 min (Trig: 0.4054*h+69.710, R2 = 0.35). The triglycerides were higher when birds were housed with 20 lm (49.68 ± 20,6) than 5 lm (43.48 ± 17.1). The difference between plasma and serum is the fibrinogen and anticoagulants factors. Thus, the proportion of cholesterol is larger in serum. Light influences the activity of birds. The utilization of 20 lx results in a major rate change of metabolism. It necessitates that more energy be spent. Consequently, the level of triglycerides increases in blood to be transformed to glucose in liver. This triglyceride may have come from the diet or lipolysis. Concomitantly, the higher metabolism produces more free radicals. Thus, the level of cholesterol is reduced in blood because cholesterol has an antioxidant effect on cells as well as steroid hormone production.

Key Words: blood, broiler, light, fibrinogen, steroid hormone

185 Effect of storage time, time of fasting, blood fractions and light intensity in the levels of uric acid and glucose to broilers. R. Nunes*[1], L. Wachholz[1], C. Souza[1], C. Souza[1], C. Eyng[1], J. Damasceno[1], J. Oxford[2], and G. Pesti[2], *[1]Universidade Estadual do Oeste do Paraná, Marechal Candido Rondon, Brazil, [2]University of Georgia, Athens, GA.*

The effect of storage time, time of fasting, blood fractions (plasma *vs* serum), and light intensity (5 vs 20 lx) was studied using uric acid and glucose blood levels in broilers. For this study 140, 45 d old, male broilers with an average weight of 3407 ± 269 g were used. The birds were fasted for 1 h and then fed for 30 min. After ingestion of feed, blood was collected every 2 h until 12 h post feeding; from the ulnar vein, using vacuum tubes with and without anticoagulant (sodium fluoride). After the blood was collected it remained at rest for 15 min and was centrifuged at 2500 rpm for 10 min, the plasma and serum were separated in eppendorf tubes. There were 5 different storage times that were used: 0; 15; 30; 60 and 120 d. Blood analysis was performed using a biochemical autoanalyzer (Flexor EL 200) with specific kits (Elitech), calibrated with standard ELICAL. Data were analyzed using the GLM procedure of SAS. Only one interaction between fasting time and light stimulation was observed in uric acid levels. For 5 lx of light, the levels of uric acid increased linearly with duration of fasting ($P < 0.008$, UA = 0.09814*H^+1.9467, R2 = 0,13). For 20 lx, the levels of uric acid showed quadratic comportment, ($P < 0.001$), with optimal fasting time of 4 h and 53 min (0.031895*H2–0.31186*H^+2.7546, R2 = 0.2). There were no interactions between storage time, time of fasting, blood fractions, and light intensity for glucose levels. The amount of glucose was different ($P = 0.007$) comparing plasma (287 ± 16.5) vs. serum (240 ± 19.2). Light at 20-lx increased, ($P = 0,006$), the level of glucose (234 ± 19.3) when compared with 5 lx (232 ± 18.7). Using regression analysis, glucose was the highest ($P < 0.001$) at approximately 87 d and 13 h of storage (−0.0013848*S2+0.24237*S+227.524, R2 = 0,43). Uric acid is an important indicator of AA utilization in broilers but can be influenced by light and fasting time. In the higher light intensity (20 lx) the birds have more stimulus and use more energy. When there is deficiency of AA being consumed due to fasting, the organism reduces the level of uric acid, until 4 h and 53 min. Posteriorly the elevation of catabolism increases the utilization of muscle protein, increasing the level of UA. The increase of blood glucose level with 20 lx, demonstrate that birds increase their energetic metabolism, to supply energy for increased activity. It is important to note that storage time of blood samples can influence the results of blood analysis.

Key Words: blood, catabolism, light stimulation, metabolism

186 Characterization and migration of cultured quail primordial germ cells from embryonic blood and gonad. S. Yakhkeshi[1], S. Rahimi*[1], M. Sharafi[1], S. N. Hassani[2], A. Shahverdi[2], and H. Baharvand[3], *[1]Tarbiat Modares University, Tehran, Iran, [2]Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran [3]Department of Developmental Biology, University of Science and Culture, Tehran, Iran.*

Primordial germ cells (PGCs), the precursors of ova and spermatozoa are ideal tools for transgenic technology. Therefore, isolation, characterization and migration of PGCs is required. The quail has been proven to be a good model in numerous fields of research, especially for biotechnology due to short generation time. In this study, initially we attempted to isolate quail PGCs from gonad (at stage 28–30) and blood (at stage 13–15) as 2 sources of these cells. The isolated PGC were cultured on feeder cells derived from chicken embryonic fibroblast. Then characterization of these cells were evaluated during the culture. Finally, for migration assay of these cells into the gonads, cultured PGCs

were injected into blood circulation of 310 recipients quail embryos. The cultured gonadal PGC proliferated about 400-times versus 100-times in blood PGC during the first 40–50 d($P < 0.05$).The identities of the PGC were confirmed using periodic acid-Schiff (PAS) staining or anti-SSEA1, β-Catenin, β-integrin, and Nanog immunofluorescence staining or reverse transcription-polymerase chain reaction for several factors related to PGC phenotype, *DAZL*, *PRDM14*, *VASA*and *Sox2*. Cultured PGCs migrated toward the embryonic gonads when they were transplanted into the blood circulate of the recipient embryo at stage 13–15 Hamburger–Hamilton (HH). Statistical analysis was performed using one-way ANOVA followed by the Tukey post hoc test. The difference between data was considered significant at P-value < 0.05. Our results suggest that cultured PGCs retain their ability to migrate and colonize the gonads of recipient embryos.

Key Words: quail embryo, primordial germ cell, migration

187 Egg cholesterol content was correlated with serum low-density lipoprotein and could be selected by sire families. X. Chen*, X. Liu, Y. Du, and Z. Geng, *Anhui Agricultural University, Hefei, China.*

Cholesterol content in egg yolk could influence embryo development. To screen proper index for cholesterol selection, at 42 wks of age, 331 female Wenchang chickens, a Chinese indigenous breed with complete pedigree records, within 24 sire families were selected as experimental group. Eggs were collected within 4 continuous days from each female bird for egg quality and yolk cholesterol content measurement. Blood was collected from brachial vein of male and female birds for serum lipid content determination. Cholesterol content was compared among female individuals and sire families by using SAS one-way ANOVA. Cholesterol content was further analyzed for its normal distribution by using univariate analysis and data from 2 tails of cholesterol content was analyzed by independent *t*-test. Partial correlation was conducted between cholesterol content and egg quality, serum lipid content by using Proc GLM. Statements of significance were based on $P < 0.05$. The egg weight was 45.16 g, shape was 0.80, shell strength was 2.97 kg/cm^2, shell thickness was 0.330 mm, yolk weight was 15.32 g, egg-white height was 3.26 mm, Haugh unit was 57.52 and cholesterol content was 45.25 mmol/L. No significant difference was observed in egg quality within the same individual bird. However, egg weight, egg shape, eggshell strength, eggshell thickness, yolk weight, egg-white height and yolk color were all distributed in a great range and significantly differed among female chickens. In sire families, egg weight was 44.81 g, shape was 0.82, shell strength was 3.07 kg/cm^2, shell thickness was 0.337 mm, yolk weight was 15.52 g, egg-white height was 3.36 mm, Haugh unit was 58.86 and cholesterol content was 45.16 mmol/L. Egg weight, eggshell strength, eggshell thickness, yolk weight, egg-white height and cholesterol content were differed significantly among sire families. Among females, high or low content of cholesterol did not affect egg quality. However, higher cholesterol content in sire families was associated with lower yolk weight ($P < 0.001$), eggshell thickness ($P < 0.001$) and higher egg-white height ($P < 0.001$). Serum total cholesterol and HDL exhibited no difference among females and males. Increase or decrease of Yolk cholesterol did not significantly affect serum lipid content in females, however, greater yolk cholesterol associated with higher serum glucose ($P = 0.001$), and lower LDL level($P = 0.002$). The heritability of cholesterol might be low in female individuals but higher in sire families. Therefore, egg cholesterol content could be directly selected first by sire families. Serum LDL might be used as a potential index for cholesterol selection.

Key Words: Wenchang chicken, egg quality, yolk cholesterol, serum lipid

188 The effect of combined monochromatic photostimulation on reproductive activities of broiler breeders. S. Zaguri, Y. Bartman*, N. A. Cohen, L. Dishon, and I. Rozenboim, *Hebrew University of Jerusalem, Rehovot, Israel.*

Artificial targeted lighting has a key role in stimulating poultry reproduction. Light perception mechanism in birds composed 2 main components: retina and extra-retinal sites. Previous works showed that photostimulation of the eye inhibits, whereas photostimulations of the brain increased reproduction activity. Differential photostimulations of retina extra retinal sites based on retina's sensitivity to green yellow wavelengths and the deep tissue penetration abilities of red wavelengths. We found that short-day exposure to green light with long day exposure to red light increases reproduction in turkey females and broiler breeder females. Furthermore we found that blue light represses expression of retina green opsin. The purpose in this study was to examine the use of innovative lighting combinations on broiler breeders flocks. 150 broiler breeder females and 15 males (Ross) at 20 wks of age divided into 5 light treatment rooms (30 females and 3 males). Light treatments: (1) control – 14hr white light, (2) Red-Green – 14 h red light and short day (6 h) green light, (3) Green-Red – 14hr green and 6hr red, (4) Red-Blue – 14hr red light and 6hr blue (480nm), (5) Blue-Red – 16hr blue light and 6hr red light. The lighting treatment was from 20 until 60 weeks of age. Data including parameters per week in each of the treatments collected for 40 weeks. Weekly data was split into 3 laying periods: A-start of production, B-peak of production and C-end of experiment, with 9/10 weeks per period (n = 9, n = 10). Analysis of the mean hen weekly data in each of those periods was subject to one way anova with the lightning treatment as a constant effect. All rearing protocols conducted according to breeder recommendations. Daily egg production and weight were recorded. Eggs were stored for one wk then placed in incubator for fertility and hatchability measurements. Monthly blood samples collected for steroids levels. At the end of the experiment, tissues were collected for mRNA gene expression. Throughout the experiment, egg production was significantly the highest in the red-blue group with an average of 84.7% ($P < 0.05$), compare with the other groups as well as Ross primary breeder recommendation of 71.9%. Significant negative linear correlation was found for layers that were raised under white or breeder recommendation light regimen between number of eggs and the layer age (r = 0.276, r = 0.325, respectively). No such correlation was found for any other light treatment ($P > 0.05$). No significant differences were detected in fertility and hatchability results. We conclude for the first time that targeted illumination can improve breeder's production in experimental model of broiler breeder flock.

Key Words: phtotostimulation, broiler

189 Yolk sac tissue in chicken embryos: The ultrastructural development during incubation. Z. Uni*, J. Dayan, and N. Reicher, *Hebrew University, Rehovot, Israel.*

The yolk sac tissue (YST) is an extra-embryonic tissue that has a central part in chick embryonic nutrition and development. The epithelial cells of the YST absorb nutrients from the yolk, digest and re-assemble them, and secrete them to the blood circulation of the embryo. Results from our lab showed (by measuring gene expression) its abilities in carbohydrates, protein and and fat metabolism as well as in synthesis of plasma proteins To follow the structural development of the tissue,

YST samples were taken at E10, E13, E15, E17, E19, and day of hatch (DOH). Observations by electron microscope (high resolution SEM) of YST samples revealed villi structures, comprised of endodermal epithelial cells (EEC) surrounding a central blood vessel. Changes are evident in EEC morphology along the incubation period: At E15, EEC are bloated, while 6 d later, at DOH, they appear shriveled. Measurements of EEC sizes showed a significant decrease from E15 to DOH ($P < 0.0001$) as the average cell perimeter decreased from 116.82 µm to 95.43 µm respectively. It was found that YST EEC contain microvilli on their apical membrane. At E15, the majority of microvilli were located at EEC borders while toward hatch a pronounced increase in their quantity was evident, with higher uniformity of microvillar distribution, covering the entire cell surface. Results of the current study demonstrate the existence of microvilli on YST cells. It might be suggested that the increasing numbers of microvilli in the YST toward hatch is due to elevated nutrient demand by the embryo. It is expected that YST development and functionality will be affected by incubation conditions by changes in YST cell dimensions, including microvilli localization and quantity.

Key Words: yolk, incubation, scanning electron microscope, embryo

190 An evaluation of the effect of two sources of sodium and exogenous phytase supplementation on diet acid binding capacity, digesta pH and blood clinical chemistry in 22-day-old broiler chickens. S. Adedokun*[1], B. Bryson[1], and M. Bedford[2], *[1]University of Kentucky, Lexington, KY, [2]AB Vista, Marlborough, United Kingdom.*

The effect of 2 sources of dietary sodium (NaCl, 100% or NaCl (38%)+$NaHCO_3$, 62%) and 4 levels of supplemental phytase (0, 500, 1,000, and 2,000 phytase units/kg; added to the NC diets) on acid binding capacity (ABC) of the diet and gizzard contents, digesta pH, and blood clinical chemistry was evaluated in 4 hundred and 32 22-d-old Cobb 500 male broiler chickens. Birds were randomly assigned to experimental diets in a RCBD with 6 replicate cages of 8 birds/cage. Orthogonal polynomial contrasts were used to determine linear and quadratic effects of phytase supplementation within each Na source. Means across Na sources were compared using specific contrasts. The PC and NC diets (NC1, NaCl only; NC2, NaCl+$NaHCO_3$) contained 0.45 and 0.35% nPP and 0.20 and 0.16% Na, respectively. The ABC of the NC1 diet was lower ($P = 0.044$; 366 vs. 405 mEq/kg) than that of the PC diet. The ABC of the NC1 diet showed a tendency to be lower ($P = 0.089$; 366 vs. 398 mEq/kg) than that of the NC2 diet. The ABC and buffering capacity (BufCap) of gizzard contents decreased linearly ($P = 0.003$) with increasing level of phytase supplementation (NaCl diets only). Excreta moisture content was higher ($P = 0.012$) by 3.5% in birds fed the NC2 vs NC1 diets. Sodium and Mg in gizzard contents were higher ($P < 0.05$) in birds on the NC2 diet compared with birds on the PC diet. Birds on the PC diet had heavier ($P < 0.05$) livers than those on the NC1 and NC2 diets but not different from birds on 2,000 phytase units. Liver weight increased ($P < 0.05$) linearly with increasing level of phytase supplementation irrespective of the Na source. Jejunal (NaCl diets only) and ileal (both Na sources) digesta pH increased ($P < 0.05$) linearly with increasing phytase supplementation. Blood alkaline phosphatase levels decreased linearly ($P = 0.006$) with increasing phytase supplementation in birds on diets with NaCl+$NaHCO_3$. Blood glucose level was higher ($P = 0.020$) in birds on NC2 diet compared with birds on the NC1 diet. Increasing level of phytase supplementation resulted in a quadratic effect ($P < 0.05$) on blood urea nitrogen (BUN) irrespective of source of Na. Both linear and quadratic effects ($P < 0.05$) of phytase supplementation on P and Ca contents of the blood were observed irrespective of Na source. Results from this study shows that most of the differences observed for different Na sources could be as a result of their effects on ABC and BufCap of the diets as seen in the gizzard contents where there was a linear decrease (with increasing phytase dose) in the ABC and BufCap in birds on diets with only NaCl.

Key Words: broiler, digesta pH, blood chemistry, sodium, phytase

191 Meta-analysis of phosphorus ileal digestibility in growing broilers: effect of dietary phosphorus, calcium and phytase supply. C. Couture*[1], R. Chiasson[1], A. Narcy[2], and M.-P. Montminy[1], *[1]Université Laval, Quebec, QC, Canada, [2]UMRBOA, Nouzilly, France.*

A key element for sustainable poultry production system is the optimization of phosphorus (P) utilization efficiency. Optimizing P utilization requires improving our capacity to predict the amount of P absorbed and retained, taking into account the main modulating factors, and a precise determination of P requirements. Given the recent consensus of expressing P availability in poultry as ileal digestible P, published data are now numerous. A meta-analysis was therefore performed to quantify the impact of the dietary forms of P (Non-Phytate P (NPP) and phytate P (PP)), of the amount of dietary calcium (Ca) and of exogenous phytase on P apparent ileal digestibility in growing broilers. A database of 77 publications, reporting 112 trials and 693 treatments was used. Dietary forms of P, namely PP, NPP from plant, from mineral, ans from animal were recalculated based on feedstuff tables. Multiple linear regression models predicting the digestible P (Total P × Apparent Ileal Digestibility coefficient, g/kg) with the experiment as random effect showed that without phytase, the AID was higher in NPP from plant (0.75), followed by NPP from monocalcium phosphate (0.69), from animal origin (0.66), from dicalcium phosphate (0.55) and from Phytic P (0.53) ($P < 0.001$; $R^2 = 0.94$)). The inherent capacity of broilers to digest PP, however, showed a linear decrease with increasing dietary Ca (Ca × PP, $P < 0.001$). When expressing NPP as the sum of plant, mineral and animal it showed a linear and quadratic effect on digestible P ($P < 0.001$) indicating that a plateau is reached. In the overall database, dietary Ca as a negative effect on both NPP (Ca × NPP, $P < 0.001$) and PP (Ca × PP, $P = 0.03$). The increase in digestible P with the addition of microbial phytase (PhytM) was curvilinear ($P < 0.001$) and showed a higher effect and a more linear response in low than in high Ca diet (PhytM × Ca and PhytM × PhytM × Ca, $P < 0.001$). The response of digestible P to PhytM also depends on the amount of substrate (PhytM × PP, $P < 0.001$). These interactions showed that phytase efficiency is higher in high PP and low Ca diets. This meta-analysis improves our understanding of P digestive utilization, with major modulating factors taken into account. The information generated will be useful for the development of robust models to formulate environmentally friendly diets for growing broilers.

Key Words: phosphorus, digestibility, calcium, phytase, broilers

192 Energy-releasing efficacy of a 6-phytase, Natuphos E, on turkey performance compared with fat reduction in corn soy diets. M. Coelho*, R. Jones, and F. Parks, *BASF Corporation, Humble, TX.*

Recent studies demonstrated that higher doses of phytase can break down most of the phytic acid and release energy from the inositol ring. The objective of this experiment was to evaluate the metabolizable energy (ME)-releasing efficacy of a 6-phytase, Natuphos E, on turkey performance compared with fat in corn soy diets. A total of 1050 one-day old BUT big 6 turkey toms were randomly allocated into 7 dietary treatments with 10 replicates and 15 birds each replicate and $P < 0.05$ was used to determine the level of significance. Dietary treatments consisted of positive control (PC) with all nutrients meet or exceed BUT big 6 turkey tom nutrition manuals; NC1 = PC- 0.17% Calcium (Ca), - 0.15% non-phytate phosphorus (nPP), −100 kcal/kg, NC2 = PC- 0.17% Ca, - 0.15% nPP, −200 kcal/kg and NC3 = PC- 0.17% Ca, - 0.15% nPP, −300 kcal/kg and phytase treatments were NC1+1000, NC2+2000 and NC3+3000 FTU/kg. Feed intake, body weight, and feed conversion ratio (FCR) were recorded at wk 3, 6, 9, 12, 16 and 20. Data were subjected to Duncan's Multiple Range Test. For the results, wk 20 body weight was 20.250, 19.572, 19.025, 18.102, 20.323, 20.508 and 20.693 kg, respectively; FCR was 2.450, 2.487, 2.556, 2.608, 2.422, 2.453, 2.430, respectively. NC1, NC2 and NC3 decreased ($P < 0.05$) body weight and increased ($P < 0.05$) FCR compared with PC.

NC1+1000, NC2+2000 and NC3+3000 FTU/kg Natuphos E, increased ($P < 0.05$) body weight and body weight gain, and decreased ($P < 0.05$) FCR compared with the NC1, NC2 and NC3, respectively. Body weight, body weight gain and FCR of NC1+1000, NC2+2000 and NC3+3000 FTU/kg treatment was equal ($P < 0.05$) to PC at wk 3, 6, 9, 12, 16 and 20. In conclusion, 1000, 2000 and 3000 FTU/kg phytase, Natuphos E, compensated, performance wise, for the reduction of 100, 200 and 300 kcal/kg, respectively, and met or exceeded ($P < 0.05$) the body weight, body weight gain and FCR of the PC. Supplementation of 3000 FTU/kg Natuphos E could compensate, performance wise, the reduction of 0.18% calcium, 0.15% available phosphorus, 0.02% sodium and 300 kcal/kg metabolizable energy in the diet.

Key Words: phytase, metablizable energy, performance, turkeys, fat

193 Evaluation of the nutritional potential of a global enzyme solution based on a multi-carbohydrase and phytase complex in laying hens. A. Bello*[1], M. Jlali[1], C. Deschamps[1], M. Ceccantini[2], R. Shirley[3], L. Jeay[4], and A. Preynat[1], *[1]Adisseo France S.A.S, Malicorne, France, [2]Adisseo France S.A.S, Antony, France, [3]Adisseo USA, Atlanta, GA, [4]Neovia, Saint-Nolff, France.*

The nutritional value of enzyme is often determined by comparing the performance of laying hens fed nutrient-adequate diet (PC) to those fed nutrient-reduced diet (NC) with and without the enzyme supplementation. A new generation of global enzyme solution, which comprise of a multi-carbohydrase (rich in xylanases and arabinofuranosidases from *Talaromyces versatilis*) and a phytase (*Buttiauxiella species*-sourced, used at superdosing level) complex (MCPC) is proposed. In the current study, the nutritional value of the MCPC in laying hens was assessed with an NC diet reduced in ME by 4%, dAA by 5%, and avP and Ca each by 0.23% point without and with the MCPC, which provides activities of 1,250 xylanase U, 860 β-glucanase U and phytase at 1,000 FTU/kg. A PC with 2,700 kcal/kg ME, 0.65% dLys, 0.35% avP, and 3.5% total Ca was also fed as one of the 3 experimental diets. Laying rate, egg mass, feed intake, FCR, and BW were measured and percentages of broken and abnormal eggs were determined in brown egg-layers (n = 1,080; 54 cages × 3 diets) from 39 to 62 weeks of age (woa). A randomized complete block design was used in the experimental set-up and data analysis was performed using Proc. Mixed of SAS. The nutrient reduction in the NC diet did not adversely affect performance and egg quality parameters compared with PC from 39 to 62 woa, except for feed intake and BW. Feed intake was increased by 1.6% and BW was reduced by 3.4% for the NC hens compared with the PC hens ($P \leq 0.05$). The MCPC completely alleviated the decreased BW of the NC hens to the similar level as the PC hens. The addition of MCPC improved laying rate, egg mass, and FCR by 2.8 ± 1.2% and the egg quality parameters by 19.8 ± 3.3% relative to both control diets ($P \leq 0.02$). These results demonstrated that laying hens fed nutrient-reduced diets efficiently supported performance with decreased BW across the 39 to 62 woa. The decreased BW of NC hens may indicate loss of body reserve as muscle, fat, and structural bone tissues. The MCPC improved overall performance and egg quality with maintained BW relative to the PC, suggesting the efficacy of the enzymes to increase energy and nutrient availability and bioavailability to support a better productivity and body reserve in laying hens. Overall, the nutritional value of the global enzyme solution, which comprised of a multi-carbohydrases and a phytase, allow for significant reduction in feed cost with increased nutrient efficiency and maintenance laying hen performance, egg quality, and welfare.

Key Words: egg, global enzyme solution, laying hen, nutrient, productivity

194 Evaluation of a new generation intrinsically heat-stable phytase at different levels of inclusion on performance and tibial ash contents of broiler chickens. S. Haldar*[1] and K. De[2], *[1]Agrivet Consultancy P Ltd., Kolkata, India, [2]Novus Animal Nutrition (India), Kolkata, India.*

It is hypothesized that if the supply of phytate P is increased then the supplemental phytase should work more efficiently. An investigation was designed to ascertain the effects of an intrinsically thermostable phytase at 500, 750 and 1000 ftu/kg on the zoo-technical performance and tibia ash content of broiler chickens. The trial was conducted for a period of 38 d in an open housing system. A total of 900 Cobb400 mixed sex chicks were assigned to 6 treatments each with 6 pens (n = 25 chicks per pen, male to female ratio was kept at 50: 50 as far as possible). Diet was formulated as per industry standard. Control diet was without phytase. NC1 diet was fomulated to contain - 0.17% Ca and Av P and the NC 2 diet was formulated to contain −0.19% Ca and Av P. The NC1 diet was supplemented with the phytase at 500 FTU/kg and the NC2 diet was supplemented with 750 FTU/kg and 1000 FTU/kg of the same phytase. Live weight, feed intake and feed conversion efficiency was recorded at 14th, 28th and 38th day. Left tibia was collected at 21 and 38 d and analyzed for total ash by ignitiona method. The data were analyzed according to GLM by one way ANOVA. Means were seperated by Tukey's B test when found significant ($P < 0.05$). The dose response of the incremental levels of phytase was determined by applying polynomial contrast. Both negative groups (T2 and T3) had lower body weight and FCR than the control group.At 14 d T5 showed better BW and FCR ($P < 0.0001$) and T4 matched the BW and FCR compared with the control group; while the highest level of phytase at T6 did not show benefits compared with lower doses. Later, at 28 and 38 d of age T4 and T5 have same BW ($P > 0.05$) and the FCR didn't show any significant result while the highest level of phytase at T6 didn't show benefits compare with lower dose. Tibia ash content was numerically lower in the NC-1 and NC-2 groups as compared with the rest of the treatment groups at 21-d of age ($P > 0.05$). At 38-d of age tibia ash content in T3 was reduced, T4 and T5 had better values than each negative group but only the T6 had same values as the control feed ($P < 0.0001$). It was concluded from the present study that Intrinsically heat stable Phytase at 500 FTU showed optimum performance for the reduction of 0.17 available P, at 750 FTU/kg showed optimum performance for the reduction of 0.19 available P and at 1000 FTU/kg showed same tibia ash as the control group.

Key Words: broiler, performance, non-phytate phosphorus, phytase, tibia ash

195 Effect of two limestone sources on ileal IP6, tibia ash and performance of broilers fed diets supplemented with two phytases. Y. Dersjant-Li*[1], R. Davin[2], T. Christensen[3], and C. Kwakernaak[2], *[1]DuPont Industrial Biosciences, Marlborough, United Kingdom, [2]Schothorst Feed Research, Lelystad, the Netherlands, [3]Nutrition Biosciences ApS, DuPont IB, Brabrand, Denmark.*

The effect of 2 limestone sources on the efficacy of 2 phytase products was evaluated in broilers during 0–21d of age. The limestone sources were a coarse (75% between 0.3 and 0.6 mm; Ca = 37.4%) and a fine limestone (<0.09 mm; Ca = 38.3%), with in vitro Ca solubility of 26 vs. 100% respectively after 30 min at pH 3. For each limestone source, positive control (PC) starter (0–10 d) and grower (10–21 d) diets were formulated (Aviagen specifications NL, 2014). Four test diets were formulated with reduced P (0.189%), retainable P (0.159%), Ca (0.199%) and Na (0.04%) contents only by exchange of MCP-P, lime-

stone, salt and diamol vs PC and supplemented with 500 or 1000 FTU/kg of either a *Buttiauxella* phytase (PhyB500 or PhyB1000) or *E. coli* phytase (PhyE500 or PhyE1000). Each treatment (10) had 8 replicates (30 Ross 308 male broilers/pen). Pelleted diets (based on corn/SBM/rapeseed meal/sunflower meal) and water were provided *ad libitum*. At 21 d, the left tibia bone was collected from 4 birds per pen and pooled per pen to measure defatted tibia ash. Ileal digesta were collected from 12 birds per pen, pooled for phytate P (IP6) analysis. BWG, FI, FCR were determined per phase and for overall 0–21d. Data were analyzed based on a 2x5 (with PC) and 2x2x2 (without PC) factorial arrangement using JMP 11 ($P \leq 0.05$ considered as significant). Coarse limestone improved ($P < 0.05$) FI (1320 vs 1295g) and BWG (1079 vs 1056g) during 0–21d and FCR during 0–10d (1.08 vs 1.09). A limestone × diet interaction ($P < 0.05$) was found for FI, BWG and FCR during 11–21d, and for FI and BWG during 0–21d, as PhyE500, PhyE1000 and PhyB500 performed worse when provided with fine limestone. Tibia ash and ileal IP6 content were not affected by limestone source. Across limestone sources, during 0–21d, PhyB1000 showed the best ($P < 0.001$) BWG, FI, FCR, tibia ash and lowest ileal IP6 content compared with other phytase treatments (1089c, 1070b, 1088c, 1031a, 1059b g for BWG; 1355c, 1306b, 1325c, 1268a, 1303b g for FI; 1.225bc, 1.221ab, 1.219a, 1.23c, 1.23c for FCR; 508d, 502bc, 505cd, 482a, 498b g/kg DM for tibia ash; 3.54a, 1.07d, 0.43e, 2.03b, 1.42c %DM for ileal IP6 content, respectively for PC, PhyB500, PhyB1000, PhyE500, PhyE1000). The IP6 reduction vs PC was 70, 88, 43 and 60% respectively for PhyB500, PhyB1000, PhyE500, PhyE1000 ($P < 0.001$). Across doses and excluding PC, a limestone and phytase interaction ($P < 0.05$) was found for 0–21d BWG, where fine limestone reduced BWG by 3.3% for PhyE and by 1.2% for PhyB. PhyB had lower ileal IP6 content than PhyE (0.75 vs 1.73% DM). In conclusion, PhyB showed a higher efficacy and was less impacted by soluble Ca than PhyE, this is most likely due to a higher IP6 degradation rate.

Key Words: limestone particle size, Ca solubility, phytase, broiler, ileal IP6 content

196 Effects of various concentrations of phytase on the performance and bone ash, of 14-day old broilers. N. Barrett*, N. Lee, and M. Persia, *Virginia Tech, Blacksburg, VA*.

Grains typically fed to broiler chickens contain phosphorus in a non-bioavailable form known as phytate. The addition of phytase enzymes to the diet have been used to improve performance when broilers are fed a diet low in non-phytate phosphorus (nPP). The current experiment was designed to determine the effects of high concentration phytase feeding on the performance and bone ash of 14-d old broiler chicks fed low nPP diets. Four treatments were used in the experiment, consisting of 10 replicate groups of 21 chicks resulting in a total of 840 Hubbard × Ross 708 chicks. The treatment groups consisted of a positive control (PC) which received inorganic phosphorus (0.45% nPP), a negative control (NC) with 0.33% nPP, the NC plus 4000 FTU of a fungal phytase, and the NC plus 10,000 FTU of phytase (DSM, Parsippany, NJ). Body weight gain (BWG: g/chick) and feed efficiency (g gain/kg feed intake) over the 14 d period were calculated. At the end of the 14 d period, 5 chicks per pen were euthanized and the right tibia was analyzed for tibia ash (g/bird). All data were analyzed using ANOVA and a Fisher's LSD test to separate means ($P \leq 0.05$). Initial body weights were similar (43.3g/chick among treatments ($P = 0.31$). Birds fed the NC diets resulted in BWG of 348.8g, lower than the PC fed birds (363.1g; $P \leq 0.05$). The addition of 4,000 and 10,000 FTU of phytase to the NC fed birds resulted in BWG of 374.4 and 378.1g, respectively. The 10,000 FTU fed birds resulted in increased BWG ($P \leq 0.05$), although the 4,000 FTU fed birds were intermediate between the PC and 10,000 FTU fed birds. There was a trend of increased feed efficiency ($P = 0.07$) with the addition of 4,000 and 10,000 FTU of phytase over both the PC and NC fed birds. Contrary to BWG, tibia ash weight was highest for the PC fed birds (1.9g), lowest for the NC fed birds (1.6g) and intermediate for the phytase fed birds (1.7g) ($P \leq 0.05$). These data suggest the inclusion of phytase at higher concentrations could further improve the performance of broiler chickens fed low nPP diets, regardless of phosphorus status of the birds.

Key Words: High phytase feeding, broiler, performance, bone ash

197 Effects of a global enzyme solution on growth performance, carcass and tibia characteristics in broilers fed corn-wheat-soybean based diets reduced in metabolizable energy and nutrients at 42 days of age. M. Jlali*[1], A. Bello[1], M. Ceccantini[4], D. Moore[2], R. Shirley[3], and A. Preynat[1], *[1]Adisseo France S.A.S, Malicorne, France, [2]Colorado Quality Research, Wellington, CO, [3]Adisseo USA, Atlanta, GA, [4]Adisseo France S.A.S, Antony, France.*

To evaluate the potential of exogenous enzymes, it is common to reduce the nutrient level of the feed and compare the performance without and with the enzyme solution in comparison to a control diet. The present study evaluated the potential of a multi-carbohydrase and phytase complex (MCPC) to compensate drastic nutrient reductions through evaluation of growth performance, carcass and tibia characteristics. Male Cobb 500 broilers (n = 1,020; 17 birds × 60 pens) were fed a common corn-based diet from 1 to 10 d and were randomly distributed to one of 5 experimental diets from 11 to 42 d of age. The diets were an adequate positive control (PC) diet and 2 negative control diets, each without (NC1 and NC2) or with the MCPC (1,250 xylanase U, 860 b-glucanase U and 1,000 FTU/kg diet). Relative to the PC, the NC1 and NC2 were similarly reduced in avP by 0.18% unit and in Ca by 0.16% unit, and were differently reduced in ME and dAA each by 5% or 7%, respectively. Growth performance (BW, BWG, FI, and FCR) were measured from 0 to 42 d of age. On d 42, 8 randomly selected birds per pen were weighed and processed for carcass yield. Also, one randomly picked bird per pen was euthanized for tibia excision. Data were analyzed using Proc GLM of SAS 9.4. Final BW, BWG 10–42 d, carcass, breast, and leg weights, tibia mineralization and strength were significantly reduced ($P < 0.0001$) with the lower nutrient specifications (NC1 and NC2) in comparison with the PC diet. However, severe ME and nutrient reduction (NC2) significantly increased ($P < 0.0001$) the FI and BWG without an improvement for FCR in comparison with birds fed NC1. The supplementation of both NC1 and NC2 diets with MCPC completely recovered ($P > 0.75$) the adverse effects of the significant reductions in energy and nutrients on growth performance (+0.1% to +0.3%), carcass (+0.6% to +0.9%), breast meat (+0.6% to +3.7%) and leg (+1.1% to +0.2%) weights of chickens relative to those fed PC diet. In addition, the tibia ash, calcium and phosphorus contents as well as tibia breaking strength in broilers fed NC diets supplemented with MCPC were fully recovered ($P > 0.70$) to the same levels as in birds fed PC diet. This study demonstrated that use of the MCPC containing a multi-carbohydrase rich in xylanase, b-glucanase, and arabinofuranosidase combined with 1000 FTU of phytase allowed to recover the performance, carcass and tibia characteristics up to −7% ME, −7% dAA, −0.18% unit AvP, and −0.16% Ca. Hence, the global enzyme solution could be an effective mean of increasing the profitability of the poultry industries by reducing the costs of meat production.

Key Words: broiler, carcass, global enzyme solution, mineralization, performance

198 Exogenous α-amylase supplementation reduces the variability of ileal digestible energy in broiler chickens fed diets with corn batches of variable protein solubility. L. Romero*[1], J. Sorbara[1], A. Cowieson[1], H. Liu[2], S. Wang[2], J. Wu[2], and A. Kluenter[1], *[1]DSM Nutritional Products, Kaiseraugst, Switzerland, [2]DSM Nutritional Products, Bazhou, China.*

This study evaluated the relationship between salt-soluble protein (SSP) of corn and the apparent ileal digestibility (AID) of complete diets in response to exogenous α-amylase supplementation in broiler chickens. Samples of 12 corn batches originating from the mid-north and northeast of China were analyzed for SSP. Six of the 12 sources of corn with a wide variation of SSP (21.1% to > 50%) were selected. Six corn-soybean-meal-based diets were formulated, including each batch of corn with or without the addition of α-amylase (Ronozyme HiStarch; DSM Nutritional Products). A total of 960 d old male Arbor Acres Plus broiler chickens with 8 replicates per treatment were randomly allocated to wired cages and fed experimental diets in 2 phases (d 0–14; d 14–28). At d 28, 4 birds selected from each cage were euthanized for evaluation of AID of starch and energy. The data was analyzed using ANOVA and the model included the effects of corn batch, α-amylase supplementation and their interaction. Significance was evaluated at $P < 0.05$. The addition of α-amylase reduced the FCR of chickens from 0 to 28 d (1.406 vs. 1.426) compared with the basal diets across corn batches, particularly from 0 to 7 d (1.049 vs. 1.078). An interaction between corn batch and α-amylase supplementation on FCR was detected only at 7 d, when the batch with the lowest SPP exhibited the greatest response to α-amylase. Neither a main effect of α-amylase nor an interaction with corn batch on AID of starch were detected ($P > 0.05$). Nonetheless, α-amylase supplementation increased ileal digestible energy (IDE) of diets from 2,915 to 2,986 kcal/kg DM across corn batches. Those increments were mainly driven by the corn batch with the lowest SSP. α-amylase supplementation reduced the variability of IDE from a CV = 5.2% to a CV = 0.7% across corn batches. Interestingly, there was a strong negative correlation ($r < -0.95$) between the initial IDE of corn and the IDE response to α-amylase, and a less strong correlation between SSP and the IDE response to α-amylase ($r = -0.54$). Dietary α-amylase supplementation has the potential to significantly reduce the variability in the energy value of corn-based diets in commercial poultry production by improving the nutritional value of poorly digestible corn.

Key Words: amylase, broiler, corn, salt soluble protein, energy

199 The benefits of using digestible AA and metabolizable energy matrix values in addition to P and Ca in broiler diets. Y. Dersjant-Li*, M. Hruby, E. White, R. Hardy, and C. Evans, *DuPont Industrial Biosciences, Marlborough, United Kingdom.*

A phytase that is highly active in the upper GIT of poultry will break down phytate quickly and more completely. Such a phytase releases P from phytate and reduces the anti-nutritional effects of phytate, contributing to dig AA and energy. This study evaluated the possibility of applying dig AA, ME, Ca, dig P and Na matrix values in broilers. A meta-analysis was done using 2 sets of data: 1) Data collected from 6 trials containing: positive control (PC), negative control 1 (NC1) and NC1+500 FTU/kg of *Buttiauxella* phytase (NC1+500FTU). NC1 and NC1+500FTU had an average reduction of 0.13% dig P, 0.16% available P, 0.16% Ca, 0.03% dig Lys and 65 kcal/kg ME, and up to 0.03% Na vs. respective PC. 2) Data collected from 3 trials with 3 treatments: PC, negative control 2 (NC2) and NC2+1000 FTU/kg of *Buttiauxella* phytase (NC2+1000FTU). NC2 and NC2+1000FTU had an average reduction of 0.15% dig P, 0.18% available P, 0.16% Ca, 0.04% dig Lys and 71 kcal/kg ME, and up to 0.04% Na vs. respective PC. Treatment means were compared by Tukey's HSD using JMP 11 (trial as a random effect). The trials were conducted in various regions globally including a semi-commercial scale trial with 700 birds/pen. The PC was formulated based on the industry standards, meeting the minimum requirements of broilers. Diets were mainly based on corn and SBM in pellet or mash form. The average Ca and dig P levels in PC diets were 0.96 and 0.42 in starter (0–10d), 0.87 and 0.38 in grower (11–21d) and 0.78 and 0.34% in finisher (22–42d), respectively. The average ME and dig Lys levels in PC diets were 2976 and 1.22 in starter, 3068 and 1.09 in grower and 3144 kcal/kg and 0.93% in finisher, respectively. For all phases, NC1 showed reduced ($P < 0.05$) ADG and increased FCR vs PC, FI was not affected. NC1+500FTU maintained the same performance vs PC (final BW of 2739, 2976 and 2966g; 0–42d FCR of 1.78, 1.65 and 1.66 respectively for NC1, NC1+500FTU and PC). At d 21, NC2 had 235g lower BW vs PC, while NC2+1000FTU improved BW vs PC ($P < 0.0001$; 785, 1046 and 1020g for NC2, NC2+1000FTU and PC, respectively). NC2 had consistently lower ($P < 0.05$) ADG, ADFI and higher FCR in all phases vs PC. NC2+1000FTU had the same performance compared with PC (final BW of 2290, 2832 and 2836g for NC2, NC2+1000FTU and PC, respectively). Data supports the use of dose-dependent AA and ME matrix values in addition to the mineral matrix while maintaining performance at the level of PC when phytase is included at 500 or 1000 FTU/kg of feed in their respective NC diets. Applying Ca, P, AA, ME and Na matrix would result in higher feed cost savings for the producer than limiting the matrix application to minerals only without impacting negatively a flock performance.

Key Words: phytase, broiler, digestible AA matrix, metabolizable energy matrix, performance

200 A global enzyme solution allows to significantly reduce metabolizable energy and nutrients while maintaining growth performance and carcass characteristics in wheat-soybean fed turkey poults. M. Jlali*[1], A. Bello[1], C. Picart[2], M. Ceccantini[3], R. Shirley[4], and A. Preynat[1], *[1]Adisseo France S.A.S, Malicorne, France, [2]Neovia, Saint-Nolff, France, [3]Adisseo France S.A.S, Antony, France, [4]Adisseo USA, Atlanta, GA.*

The objective of this study was to investigate if supplementation with a global enzyme solution combining a multi-carbohydrase and phytase complex (MCPC) might alleviate the negative effects of energy (ME), digestible amino acid (dAA) and mineral deficiency on performance and carcass characteristics in turkey poults from 1 to 120 d of age. The design for the current experiment included 3 dietary treatments with a positive control diet (PC), a negative control (NC) reduced in ME, dAA, AvP, and Ca by −4%, −5%, −0.18% unit, and −0.16% unit, respectively, and a dietary treatment that included a MCPC containing xylanase, β-glucanase, and arabinofuranosidase combined with 1000 FTU of phytase in addition to the NC. Each treatment was assigned to 12 replicate pens with 20 birds each for a total of 720 turkey poults (Grade Maker). The diets were fed in 6 phases (1 to 21d, 22 to 42d, 43 to 63d, 64 to 84d, 85 to 105d, and 106 to 120d) according to the breeder recommendations. Body weight (BW) and feed intake (FI) were measured at the end of each phase, and body weight gain (BWG) and feed conversion ratio (FCR) were calculated. On d120, 5 randomly selected birds per pen (60 animals per treatment) were used for carcass yield determination. Data were analyzed using Proc GLM of SAS. The reduction in ME and nutrients in the NC diet led to a significant reduction in final BW and BWG (−7.5%; $P < 0.0001$) and increased the FCR by 20 points (+8.4%; $P < 0.0001$) compared with the PC. Moreover, the carcass and breast yields were also degraded for birds fed NC by 1.4 and 4.5% units, respectively ($P < 0.0001$). The inclusion of MCPC in the NC

diet fully restore the BW, BWG, carcass and breast yields to the same levels as the turkey poults fed PC diet, and also significantly improved ($P < 0.0001$) the FCR compared with NC diet. The results demonstrate that MCPC used as a global enzyme solution has the capacity to restore the performance and carcass characteristics suggesting the possibility to lower feed cost by the reduction of energy, amino acids, calcium and phosphorus in the feed, while maintaining performance of turkey poults.

Key Words: carcass, enzyme solution, performance, turkey

201 Effect of a NSPase enzyme, Natugrain TS, on feed passage rate, jejunum viscosity, energy release and performance on broilers fed corn/soy diets processed at variable conditions. M. Coelho*[1] and A. Troescher[2], *[1]BASF Corporation, Humble, TX, [2]BASF SE, Lampertheim, Germany.*

The objective of this study was to evaluate the efficacy of a NSPase, Natugrain TS (TS) on feed passage rate (FPR), jejunum viscosity, energy release and performance on broilers fed mash and pelleted corn/soy diets processed at variable conditions for 21 d. A total of 960 male Cobb 500 broilers were used in a randomized complete block design (8 birds/cage × 6 feed processing conditions × 2 TS doses × 10 replicates), and $P < 0.05$ was used to determine the level of significance. Feed processing conditions (FPC) were mash, pelleted at 70C, 78C, 81C, 89C and 94C at 45 s conditioning. Natugrain TS doses were 0 and 100 g/MT. Resistant starch (RS) and protein (RP) (assayed with enzymatic hydrolysis), Jejunum viscosity (with a Brookfield digital viscometer), feed passage rate (FPR) (with fluorescent marker), apparent ileal digestibility of energy (IDE) (with a TiO_2 marker) and bird performance at 21 d were measured. Data were subjected to ANOVA and orthogonal contrasts were used to determine linear and quadratic responses to feed processing conditions. Pelleting at 89 and 94C had higher ($P < 0.05$) RS than mash, 70, 78 and 81C (5 and 12% versus 2, 3, 3 and 4%). Pelleting at 89 and 94C had higher ($P < 0.05$) RP than mash, 70 and 78C (4 and 7% versus 1, 2 and 2%). Birds fed pelleted feed at 94C without TS had a higher ($P < 0.05$) jejunum viscosity (4.71 mPa.s) than mash, 79 and 78C (3.83, 3.91 and 3.97 mPa.s). Birds fed pelleted feed at 94C with TS had a higher ($P < 0.05$) jejunum viscosity (2.44 mPa.s) than mash, 70 and 78C (2.31, 2.38 and 2.36). The addition of TS at 100 g/MT decreased ($P < 0.05$) the jejunum viscosity across FPC on avg. by 40%. Birds fed pelleted feed at 94C with TS had a lower ($P < 0.05$) FPR than birds fed mash and 70C. Birds fed pelleted feed at 89C with TS had a higher ($P < 0.05$) IDE than birds fed mash, 70, 78, 81 and 94C .TS decreased ($P < 0.05$) the FPR in all FPC. Birds fed pelleted feed at 89C without TS had a higher ($P < 0.05$) Weight gain (900g) than mash, 70, 78, 81 and 94C (837, 863, 878, 886 and 878g). Birds fed all FPC with TS had higher ($P < 0.05$) 0–21d weight gain versus birds fed processing conditions without TS. In summary, pelleting improved gut conditions and bird performance up to 89C, and Natugrain TS further improved gut conditions and bird performance.

Key Words: NSPase, metabolizable energy, jejunum viscosity, feed passage rate, broilers

202 Response of broiler chickens to xylanase and butyrate supplementation. G. Gonzalez-Ortiz*[1], K. Vienola[2], S. Vartiainen[2], J. Apajalahti[2], and M. Bedford[1], *[1]AB Vista, Marlborough, United Kingdom, [2]Alimetrics, Espoo, Finland.*

The application of carbohydrases in cereal-based poultry diets may contribute to the release of oligosaccharides in the gut, which may effectively work as prebiotics fermented to butyrate and improving animal performance. A 2 × 2 factorial experiment was used to evaluate the effect of xylanase and butyrate supplementation on performance, intestinal fermentation and histomorphology in broiler chickens. Three hundred eighty-four Ross 308 broiler chicks (1-day-old) were allocated to 4 experimental treatments: CTR (control diet), XYL (CTR diet supplemented with 16,000 BXU/kg of xylanase), BUT (CTR diet supplemented with 1 kg/t sodium butyrate) and XYL+BUT (CTR diet supplemented with both xylanase and butyrate). Each treatment had eight replicates of 12 animals. Starter and grower diets, based on wheat and soyabean meal, and water were available ad libitum. Body weight gain (BWG) and feed intake (FI) were measured from 0 to 42 d and feed efficiency was corrected for mortality (FE). The profile of short-chain fatty acids (SCFA) in the duodenum, jejunum, ileum and ceca digesta on d 21 and 42 were analysed. Villus height (VH), crypt depth (CD) and the villus to crypt (VH:CD) ratio from the ileal tissue on d42 were additionally evaluated. Statistical comparisons were performed using a two-way ANOVA (JMP Pro 12). Additionally the principal component analysis (PCA) of the SCFA was performed to determine the influence of the different factors (intestinal section and treatment). No interactions were observed in any of the parameters measured. Xylanase supplementation improved 42-d FE by 5 points ($P = 0.006$). The main effect of XYL was observed in the starter period, when BWG and FE were improved ($P < 0.05$). Butyrate reduced FI in the starter period ($P = 0.034$) leading to lower BWG (P = 0.018). Butyrate did not affect 42-d FE. No treatment effect was observed on any of the major SCFA profiles in any of the intestinal compartments measured on d 21, but on d 42, the butyric acid concentration in the duodenum was higher with the XYL+BUT diet ($P < 0.05$) compared to all the other treatments. Of the variability in SCFA across all the intestinal sections, 55% and 64% was mostly explained in the PCA 1, the intestinal region being the most important factor rather than the experimental treatments. XYL diet did not influence histomorphology but the VH:CD ratio was increased with BUT supplementation (8.7 vs. 10.1; $P = 0.005$). Supplementation of broiler diets with xylanase influenced performance, which may be due to a reduction in digesta viscosity, better utilization of nutrients and xylo-oligosaccharides release. Addition of butyrate increased the VH:CD ratio but did not support better performance.

Key Words: xylanase, sodium butyrate, performance, histomorphology, fermentation

203 Effect of protexin with and without commercial enzyme on performance and mucosal enzyme activity of broilers duration starter period. F. Ahmadi* and K. M. Arabi, *Islamic Azad University, Sanandaj, Kurdistan, Iran.*

This study was carried out to investigate the effect of protexin with and without commercial enzyme on performance traits and mucosal enzyme activity of broilers duration starter period. A total of 160 male chicks were distributed according to a completely randomized experimental design (CRD), with 4 treatments with 4 replicates of 10 birds in each. The experimental treatments were T1) control (basal diet), T2) basal diet supplemented with 0.12 mg protexin/kg, T3) basal diet supplemented with 0.10 mg enzyme, and T4) basal diet supplemented with the combination of protexin and commercial enzyme with the mention level (the amount of protexin and enzyme selected based on company recommendation). Birds received as adlib to feed and water during the time of study. Live Body weight (LBW), feed intake (FI) and feed conversion ratio (FCR) was recorded AT 7, 14, and 21 d of old. On d 21, 4 birds (one bird per replicate) were individually weighed and sacrificed. To evaluate the mucosal enzyme activity, removed segments of duodenum and jejunum of the small intestine and then stored in −80°C

until time of measuring ALP, amylase and lipase activity. Results indicated that the birds fed basal diet supplemented with combination enzyme and protexin (T_4) had higher LBW ($P < 0.05$) and lower FCR ($P < 0.05$) than control and other treatment. Also, the level of amylase and lipase activity improved ($P < 0.05$) in the birds that fed basal diet supplemented with protexin and enzyme in comparison with control. In conclusion, the results of the present research showed that the blend of protexin and enzyme could improve performance traits and increased level of mucosal enzymes at the duodenum and jejunum in the small intestine of broilers duration starter period.

Key Words: broiler, performance, enzyme, mucosal

204 Effect of phytases on performance, nutrient digestibility and blood profiles of meat-type chickens fed low-density diets.

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The objective of this study was to study the impact of *Aspergillus niger* (AN) and *E. coli* phytase on performance and digestibility of nutrients of colored slow-growing meat type chickens fed diet containing submarginal protein and/or energy levels. A total of 420, day-old chicks were divided into 7 groups (5 replicates of 12 chicks/group). The control fed diets with the following crude protein (CP, %) and metabolisable energy (ME, kcal) contents in starter (1–35 d), grower (37–56 d) and finisher (57–64 d) periods: 21.2/2947; 19.6/3023; 18.0/3100. The 3 low-CP groups fed a diet with similar ME and lower (– 1%) CP; the low-CP unsupplemented group (low-CP_uns) fed the diets without supplementations; the low-CP phytase groups fed the low CP diet with 500 U/kg of an AN or 500 FTU/kg of an *E. coli* phytase. The other 3 groups fed a low CP and ME diet (low-CPME; −1% CP, −100 kcal than the control) and were supplemented as low-CP groups. Low-CP and low-CPME diets reduced ($P < 0.01$) feed and protein intake, protein and metabolizable energy conversion ratios than the control. The data were analyzed was 2-way factorial design (2 types of diets by 2 types of phytase besides the positive control). The results indicate that phytase reduced ($P < 0.01$) feed, protein and energy intake and *E. coli* phytase had a stronger ($P < 0.01$) effect than fungal. Low-CP and low-CPME diets reduced ($P < 0.01$) nitrogen in the excreta than the control. The use of phytases increased ($P < 0.01$) dry matter, crude fiber and CP digestibilities and ash retention. Excreta nitrogen was reduced ($P < 0.01$) due to use of phytases. Low-CP diet decreased ($P < 0.01$) the intestinal length than the control group. The use of phytases increased ($P < 0.05$) the abdominal fat and the levels of Ca and P ($P < 0.01$) in broiler plasma and decreased ($P < 0.01$) the blood cholesterol in comparison to the unsupplemented group. In conclusion, the use of phytase, independently from its source, allows reducing the protein and energy content of Ca and P adequate -diets for colored broilers and decreased nitrogen pollution.

Key Words: phytase, protein, energy, growth, digestibility

205 Water consumption and fates in modern meat-type chickens under commercial conditions. D. Aldridge*, C. Scanes, and M. Kidd, *University of Arkansas, Fayetteville, AR.*

Water is a critically important nutrient. There is, however, little data on the consumption and fates of water in today's meat type birds. The present study examines water consumption using data from 14 flocks (straight run Cobb 500) at the University of Arkansas Savoy Applied Research Facility between 2015 and 2017 and 13 flocks between 2009 and 2010. Water consumption increased in a linear manner in the first 4 weeks. Thereafter, water consumption per bird per day slowed and then plateaued before marketing. The timing of the plateauing was earlier in 2015–2007 birds than 2009 and 2010. It was calculated that water consumption was 7.99 ± (flocks n = 14) SEM 0.289 L per bird for flocks between 2015 and 2017 excluding 2.89 ± 0.017% losses from leakage/flushing. This compares to 8.18 L between 2009 and 2010. There was increases in both water consumption and house humidity during the summer than winter. The calculated water consumption (2015–2017) was equivalent to 2.73 L per kg or 1.54 L per kg feed. These compares to 3.51 L per kg or 1.76 L per kg feed reported for the early 1980s (Pesti et al., 1985. Poult. Sci. 64:803–808). Water consumption for today's meat chickens was greater that of the 1980s but less if expressed per unit bodyweight or per unit of feed. Consumption per unit body weight were even closer when expressed per metabolic weight. Sources of water for poultry and other animals are drinking water, moisture in feed and metabolic water. It was estimated that water intake by growing broiler chickens (9.81 L) is 74.3% of drinking water with remainder being moisture in feed (7.5%) and metabolic water (18.3%). Based on the input and the average weights of chickens marketed, the outputs were estimated as 79.0% evaporative loss (respiratory, cutaneous and excreta), marketed birds (18.9%) and increases in water in litter (2.0%). The total evaporative loss translates to 145,250 L per flock for a 20,000-bird broiler house. While water dynamics of today's meat time birds have been characterized, much remained to be elucidated by both basic and applied research. For instance, what is the relative importance of evaporative losses from respiration, skin and excreta.

Key Words: water, broiler

206 A feed restriction paradigm that may improve gait soundness in grow-out ducks, Part 1: The good news. G. Fraley*[1], S. Fraley[1], L. Porter[1], A. Bentley[1], L. Van Blois[1], N. Prihoda[1], B. Hargis[2], G. Tellez-Isaias[2], C. Vuong[2], Z. Tucker[3], and D. Shafer[3], *[1]Hope College, Holland, MI, [2]University of Arkansas, Fayetteville, AR, [3]Maple Leaf Farms Inc, Leesburg, IN.*

Like other poultry species, culling of Pekin ducks is often due to gait abnormalities caused by more rapid muscle growth relative to the skeletal system. Previous studies have shown that duck leg bones grow exponentially in terms of mineral deposition during the first 2 weeks of life. Therefore, we hypothesized that a slight food restriction would reduce muscle mass development sufficiently to allow the skeletal system to more fully develop. Our first study demonstrated that an 85% pair-fed feed restriction during d 1 – 14 post hatch could allow ducks to obtain target weight by 32 d of age, but with a reduced pectoralis major mass. In this study we hypothesized that feed restriction following muscle satellite cell development after d 5 would allow full growth of the breast meat. To accomplish this goal, ducks were pair-fed to *ad lib* controls 85% daily feed intake from d 1 – 14 (FR85%1–14, n = 4 pens, 25 ducks per pen) or from d 5 – 14 (FR85%5–14, n = 4 pens, 25 ducks per pen) and compared with ad lib controls (n = 4 pens, 25 ducks per pen). All data were analyzed by ANOVA with a Fisher's PLSD *post hoc* test. Gait analyses showed that the FR85%5–14 group showed increased gait width and reduced metatarsal adduction compared with the other 2 treatment groups, traits thought to be associated with reduced lameness in ducks. Although both FR groups showed reduced body weight ($P < 0.01$) on d 14 and 21, both FR groups gained sufficient weight to achieve target weights by d 28. As we showed previously the FR85%1–14 group showed reduced ($P < 0.05$) pectoralis mass compared with controls, however the FR85%5–14 showed no differences compared with controls. No differences in offal weight were observed among the 3 treatment groups, nor were differences observed in bone ash at d 14 or 35. Finally, no differences were observed in the feed conversion ratio among the 3 groups over the 35 d study. Our observations suggest that this partial feed restriction may allow for skeletal development that may lead to a reduced occurrence of gait abnormalities in meat ducks without affecting production parameters.

Key Words: lameness, food restriction, food intake, growth rate, intestinal mucosa

207 A feed restriction paradigm that may improve gait soundness in grow-out ducks, Part 2: The challenges. A. Bentley[1], L. Porter[1], L. Van Blois[1], N. Prihoda[1], C. Vuong[2], B. Hargis[2], G. Tellez-Isaias[2], Z. Tucker[3], D. Shafer[3], S. Fraley[4], and G. Fraley*[1], *[1]Hope College, Holland, MI, [2]University of Arkansas, Fayetteville, AR, [3]Maple Leaf Farms Inc., Leesburg, IN, [4]South Crossing Veterinary Center, Caledonia, MI.*

We hypothesized that feed restriction following the muscle satellite cell development after d 5 would prevent full development of breast meat. To test this, ducks were pair-fed to ad lib controls 85% daily feed intake from d 1 – 14 (FR85%1–14, n = 4 pens, 25 ducks per pen) or from d 5 – 14 (FR85%5–14, n = 4 pens, 25 ducks per pen) and compared with ad lib controls (n = 4 pens, 25 ducks per pen). All data were analyzed by ANOVA with a Fisher's PLSD *post hoc* test. The FR85%5–14 did show similar production standards to ad lib controls, but with improved skeletal development, however we wanted to further assess the effects of FR on immune competence. To determine if the partial feed restriction would have an impact on intestinal epithelial tight junction integrity, we treated ducks on d 7, 14, 21, 28, and 35 with 8.32 mg/kg FITC-d in water per os and blood samples were obtained via the leg vein one hour later. Serum samples were analyzed for presence and quantification of FITC-d using a spectrometer at 485nm/528nm ex/em. Feed restriction elicited an increase in gut leakiness as determined by elevated ($P < 0.05$) levels of serum FITC-d. We further wanted to elucidate whether feed restriction would impact the ducks' acquired immune response. To determine this we vaccinated all ducks with ~10^8 inactivated *Salmonella enteritidis* in mannosylated chitosan adjuvant intramuscularly on d 14 and boosted on d 26. Anti-*S. enteritidis* specific IgY responses were assessed by ELISA from serum collected at 14, 28, and 35 d. Although all ducks showed an increase humoral immune response to the *S. enteritidis*, both feed restricted groups showed reduced IgY production compared with ad lib controls. Our data suggest that although the FR85%5–14 feed restriction paradigm may reduce gait abnormalities without affecting production rates, some challenges exist due to increased gut leakiness or decreased acquired immune activity. Future studies will look at altering the feed restriction milieu to ameliorate these challenges.

Key Words: lameness, food restriction, intestinal mucosa, acquired immunity, leaky gut

208 The in ovo critical period for somatotrophic axis elevation by green light photostimulation. L. Dishon*[1], N. A. Cohen[1], Y. Bartman[1], S. Zaguri[1], T. Porter[2], and I. Rozenboim[1], *[1]Hebrew University of Jerusalem, Rehovot, Israel, [2]University of Maryland, College Park, MD.*

In ovo green light (GL) photostimulation of meat type birds elevated body weight and muscle growth at market age. The mechanism of this phenomenon was likely due to an elevation of the somatotropic axis activity. The objective of this study was to define the in ovo critical period for stimulating the somatotropic axis by GL photostimulation. Two hundred equal weight fertile broiler eggs (Cobb 500), were divided into 4 in ovo treated groups: 1. Incubated under dark conditions (Negative Control n = 60), 2. GL photostimulated from embryonic d 0 (ED0) until ED20 (hatching day) (positive control n = 60), 3. GL photostimulated between ED10 and ED20, (n = 50) and 4. GL photostimulated between ED15 and ED20 (n = 30). At ED10 and every other day until ED20, 10 eggs from each treatment group were sampled. Eggs were opened, blood samples were drawn for GH ELISA assay, and hypothalamus, liver, and breast muscle samples were collected for analysis of mRNA levels for GHRH, GHR (growth hormone receptor) and IGF-1 (insulin-like growth factor 1), by real-time PCR. After finding no significant interaction between treatment and ED, all statistical analyses were conducted with the JMP software using one-way ANOVA. In ovo GL photostimulation from ED0 caused a significant elevation ($P < 0.05$) in plasma GH levels (between ED14-ED20) of between 35% and 100%, compared with negative control. Hypothalamic GHRH mRNA levels significantly increased by 30% (on ED16 and ED20), and both liver GHR (on ED12 and ED16 to 18) and IGF-1 (on ED16–18) mRNA levels were significantly elevated by 20–100%, compared with the negative control. In-ovo GL photostimulation from ED10 showed positive effects (compared with the negative control) on GH plasma levels ($P < 0.05$), with no effect on mRNA levels ($P > 0.05$). Green light photostimulation from ED15 showed elevation ($P < 0.05$) in somatotropic axis activity similar to the positive control group. In ovo, green light photostimulation of broiler embryos, from ED15 to ED20 significantly elevated somatotropic axis activity similar to the positive control group. We suggest that the critical period for GL photostimulation acceleration of somatotropic axis is between ED15 and ED20.

Key Words: broiler, development, in ovo photostimulation, somatotrophic axis

209 Efficacy of sodium bisulfate or sodium chloride for reducing broiler house floor microbial populations. J. Payne*[1] and S. Watkins[2], *[1]Jones-Hamilton Co., Coweta, OK, [2]University of Arkansas, Fayetteville, AR.*

Sodium bisulfate is often applied to acid shock poultry house dirt floors following litter removal. Some farms utilize sodium chloride (salt) as a floor treatment. A field study was conducted to compare the efficacy of sodium bisulfate and salt for reducing broiler house floor microbial populations following litter removal. Commercial broiler houses were chosen as the test sites. Floor plots (0.6 m × 3 m) were established under feed and water lines throughout the house and treatments were assigned using a complete randomized block design. In trial 1, treatments consisted of (1) control; (2) sodium bisulfate at 45 kg/93 m^2 (100 lbs/1000 ft^2); (3) sodium bisulfate at 68 kg/93 m^2 (150 lbs/1000 ft^2); and (4) Farmer's Coop fine rock salt at 68 kg/93 m^2 (150 lbs/1000 ft^2). There were 4 replicate plots per treatment. In trial 2, treatments consisted of (1) control; (2) sodium bisulfate at 68 kg/93 m^2 (150 lbs/1000 ft^2); and (3) Farmer's Coop fine rock salt at 68 kg/93 m^2 (150 lbs/1000 ft^2). There were 5 replicate plots per treatment. Floor plots were aseptically swabbed using a sterile cellulose sponge pre-treatment and at 24 h and 72 h post-treatment. Samples were cultured to determine populations of total aerobic bacteria, *E. coli*, total coliforms, yeast, and mold. Floor plot surface pH was also measured. Additionally, samples in trial 2 were cultured for the presence of *Staphylococcus* spp. Data were analyzed using the ANOVA procedure of SAS. In both trials, salt had no effect on surface pH and populations of total aerobic bacteria and mold and limited to no impact on yeast populations. Sodium bisulfate showed significant reductions in floor pH and populations of total aerobic bacteria, yeast, and mold in both trials. *E. coli* and total coliform counts were reduced over time regardless of treatment. *Staphylococcus* spp. presence in samples was reduced from 100% pre-treatment to 80%, 60%, and 0% positive 72 h post-treatment in control, salt, and sodium bisulfate treatments, respectively. Sodium bisulfate proved effective for reducing microbial populations on a broiler house floor while salt showed limited to no effect.

Key Words: bacteria, sodium bisulfate, pathogens, pad treatment, sanitation

210 Effects of PLT application in nonbrood chamber on broiler spatial distribution, production performance, and ammonia emission. H. Li*[1] and J. Payne[2], *[1]University of Delaware, Newark, DE, [2]Jones-Hamilton Co., Coweta, OK.*

Use of acid-based litter treatments is an important tool in the management of built-up litter and ammonia control. The deleterious effects of ammonia on bird performance, health, and carcass quality have been well documented. Several commercial products meet the demand on ammonia control. These products are used primarily in the brood chamber during all seasons to address and assist with such issues as high fuel costs, short lay-outs between flocks, prolonged reuse of litter, wet litter conditions, and ammonia-related stress. In addition, poultry welfare standards recommend that birds be raised under minimum ammonia levels, generally less than 25 ppm. Litter treatments have played a vital role in helping to achieve these goals particularly during the brooding period. Litter amendments have been applied by some broiler growers to the entire poultry house including the nonbrood chamber. However, the benefits of the nonbrood chamber application have not been documented. A field demonstration project was conducted to evaluate the effects of PLT (sodium bisulfate) application in the nonbrood chamber on broiler spatial distribution and production performances. Two identical broiler houses were used to raise 3 flocks of broilers over a 7-wk growout period. Each house measured 57 × 37 ft (length × width) and was divided into 2 chambers with a center brood curtain and brood board. Separate water meters, feed scales, and bird scales were used in both brood and nonbrood chambers. PLT was applied to the built-up litter on day −1 in the brood chamber in both houses and on d 6 in the nonbrood chamber of the treatment house. The brood curtain and board were removed and whole floor access was provided to chicks on d 7. The bird spatial distribution on the whole floor in the control and treatment house was evaluated by comparing the water and feed consumptions and bird scale hit numbers. Growth rate, feed conversion ratio (FCR), and ammonia emission rate were also evaluated by using paired comparison. The results showed that water consumption and bird scale hits were more evenly distributed in the houses with nonbrood chamber PLT application ($P < 0.05$). Birds had heavier weight and better feed conversion with non-brooding PLT application. Ammonia emission was

reduced by 10% in the nonbrood treated house ($P < 0.05$). This project confirmed that whole house PLT application improved the bird spatial distribution and production performances.

Key Words: litter treatment, PLT, ammonia, distribution, production

211 Dynamics of broiler breeder growth and its effect on egg production and egg weight of Ross 308 AP (AP95): Commercial database. F. Tovar[2] and E. Oviedo-Rondón*[1], *[1]North Carolina State University, Raleigh, NC, [2]Universidad del Tolima, Ibague, Tolima, Colombia.*

The growth patterns of pullets have been linked to egg production of several broiler breeder genetic lines. Little information is known about performance of Ross 308 AP (AP95) parent stock, genetic line developed for the Latin American market. Data from broiler breeder flocks of Latin American companies were analyzed for growth and reproductive indicators. This data was recorded between 2015 and 2017 including 49 breeder flocks representing 1'043.017 hens. Flocks were classified by an automatic classification tree analysis into superior and inferior cumulative hen-house egg production (HHEP) at 53 wk of age. All statistical analyses were conducted in JMP 13. Ross AP95 laid on average of 149.9 and 140.4 eggs/hen, for superior (9 flocks) and inferior groups (18 flocks), respectively. In 29 wk of egg production (53 wk of age) the superior group had 13.1 eggs/hen more than indicated in the performance objectives booklet for this line and the inferior 3.6 eggs/hen more than the standard. The weekly average BW and BW gain was compared by one-way ANOVA, detecting differences on growth during rearing between superior and inferior HHEP. The BW observed at 2 wk was 238 g for superior HHEP *vs*. 276 g for inferior ($P < 0.001$), at 3 wk 368 *vs*. 397 g ($P < 0.05$), 843 *vs*. 872 g at 8 wk ($P < 0.05$), 1,218 *vs*. 1,250 g at 12 wk ($P < 0.05$), 1,475 *vs*. 1,555 g at 15 wk ($P < 0.01$), and at 16 wk 1,588 *vs*. 1,663 g ($P < 0.01$), respectively. The BW for the superior group during these weeks was below the recommendations indicated in 2017 guideline for this genetic line. These differences were also detected on average daily gain between superior and inferior HHEP groups for each wk, at 2 wk of age ($P < 0.01$) 12.1 g/d *vs*. 16.9 g/d, 12.4 *vs*. 14.2 g/d at 8 wk ($P < 0.05$), 12.3 *vs*. 14.7 g/d at 12 wk ($P < 0.05$), 6.9 *vs*. 17.9 g/d at 15 wk ($P < 0.001$), and 36.5 *vs*. 24.0 g/d at 24 wk, respectively. Negative and moderate linear correlations ($r = -0.36$ to -0.45) between BW during rearing and HHEP at 31 and 53 wk were observed ($P < 0.05$). The BW at 5 wk of age had a quadratic response ($P < 0.001$) on HHEP at 30, 31 ($R^2 = 0.59$), 44 ($R^2 = 0.65$) and 53 wk of age ($R^2 = 0.53$). The BW at 6 wk of age had similar effect ($P < 0.01$) on HHEP at 30 ($R^2 = 0.37$), 31 ($R^2 = 0.36$), 44 ($R^2 = 0.40$) and 53 wk of age ($R^2 = 0.42$). Significant relationships ($P < 0.05$) were observed between BW before the onset of egg production and egg weight at 26 wk, being positive linear at 19 ($R^2 = 0.75$) and 23 wk of age ($R^2 = 0.70$) and quadratic at 24 wk of age ($R^2 = 0.82$). In conclusion, based on a commercial database of Ross AP95 parent stock flocks, there is a relationship between achieving optimum BW and average daily BW gain during rearing with HHEP and initial egg weight.

Key Words: pullet, broiler breeder, growth, reproductive performance, egg production

212 Evaluation of environmental and physiological parameters during Ventilation Shutdown in turkey hens. K. Anderson*[1], K. Livingston[1], S. Shah[1], M. Martin[2], R. Malheiros[1], and K. Eberle-Krish[1], *[1]North Carolina State University, Raleigh, NC, [2]North Carolina Department of Ag and Consumer Services, Raleigh, NC.*

In 2015, the poultry industry lost 7.6 million breeder and commercial turkeys to the worst highly pathogenic avian influenza outbreak in US history. Timely depopulation was identified as a critical measure to contain the outbreak. Approved depopulation methods were quickly overwhelmed resulting in prolonged suffering of infected birds. The objective was to evaluate the effectiveness of ventilation shutdown (VSD) and derivatives for mass depopulation in turkeys by assessing electroencephalograms (EEG) as well as environmental and physiological parameters. Two 16.0 ft^3 chambers were constructed to represent industry standards for per bird spacing within turkey facilities. The treatments included VSD and its derivatives, VSD+Heat (VSDH) and VSD+CO_2 ($VSDCO_2$), which were repeated 4 times each with one bird per chamber. EEG electrodes were attached to monitor the bird's brain activity. Data was analyzed with Proc GLM for chamber temperature, relative humidity (RH), and CO_2 concentration. The Slope of the EEG were calculated on the absolute mV.s regression analysis then the Slopes were compared using Tukeys in JPM. All pairwise comparisons were separated using LS Means. No significant difference was found for chamber temperature, RH, and CO_2 concentration at the start; temperature and RH ranged from 77.3 to 81.8°F and 25.1 to 34.0%, respectively. The ending chamber temperature was highest for VSDH at 141°F ($P < 0.0001$) and lowest for $VSDCO_2$ at 87.5°F. Relative humidity was significantly higher ($P < 0.0004$) in VSD at 66.0% compared with VSDH and $VSDCO_2$ at 34.8 and 33.5%, respectively. The starting core body temperature (CBT) for all treatments averaged 105.4°F. At time of death (TOD), the CBT was highest ($P < 0.0001$) for VSD (114.4°F) and VSDH (114.5°F). The time to reach TOD was longest ($P < 0.0001$) in VSD at 270.5 min followed by VSDH at 108.4 min; $VSDCO_2$ was shortest at 23 min. Hens in the VSD, VSDH and $VSDCO_2$ treatments spent 7.1, 13.0, and 7.4% of the time in an insensible (below 0.01mV) range, respectively. In turkeys, VSD takes significantly longer to TOD than its derivatives. The EEGs indicate sensibility until just before TOD for VSD, VSDH, and $VSDCO_2$.

Key Words: turkey, electroencephalogram, depopulation, ventilation shutdown

213 Partial slotted flooring system for market turkeys. S. Noll*, K. Janni, B. Hetchler, G. Furo, J. Brannon, J. V. Thomas, and C. Cardona, *University of Minnesota, Saint Paul, MN.*

Advantages exist for livestock and poultry production systems where wastes can be physically separated from animals and removed on a regular basis. Lower ammonia concentrations and reduced energy and bedding inputs have been noted. In turkey production systems, concerns with non-bedded flooring include development of carcass defects and leg problems. Bedding use in a turkey barn carries substantial disease introduction risk due to movement of bedding, equipment and personnel associated with the bedding process and litter maintenance. The research objective was to determine if substitution of some of the bedding with slotted flooring (SF) will impact market turkey tom rearing. Two experiments (Exp. 1 and 2) were conducted to assess live performance, carcass quality and well-being of market toms reared in pens with 25% of the bedded floor area replaced with SF from 5 to 18 wks of age. Six treatments included Control (C, 100% bedding) and 5 treatments with different types of commercially available SF. The 5 flooring materials (slat opening size and shape) were: Double L Classic Red Rooster (1.9x6.4 cm rectangular); SW Ag Plastics Dura-Slat STO (2.8 cm square); SW Ag Plastics Dura-Slat ST (2.8 cm square); Tenderfoot (2.2x5.5 cm rectangular); and Tenderfoot (2.5 cm square). Wood shavings bedding was used in all pens starting with fresh bedding for Exp. 1 and then used bedding for Exp. 2. Treatments were randomly

allocated to 2 replicate pens with 50 toms (Large White, Hybrid Converter) per pen. Studies were conducted during consecutive fall seasons. Measurements included live performance, litter moisture and scores for footpad dermatitis, feather cleanliness, and gait. Carcass quality was only assessed in Exp. 1. Pen was the experimental unit and significance was determined with ANOVA and mean testing. No consistent differences in bird performance, gait score, litter moisture or carcass breast defects (Exp. 1) were noted for flooring type ($P < 0.05$). At 18 wks, a more severe composite footpad score was observed for SW Ag Plastics Dura-Slat STO ($P < 0.05$ in Exp. 1 and $P < 0.10$ in Exp.2) in comparison to C. The same flooring showed an increased feather score (dirtiness) compared with all other treatments in Exp. 1 ($P < 0.10$) and compared with all other SF in Exp. 2 ($P < 0.05$). The studies indicate that it is possible to utilize a partially slotted floor system for rearing commercial market toms. Funding for this project was provided by the Minnesota Department of Agriculture through the University of Minnesota Response to Avian Influenza Project.

Key Words: turkey, flooring, litter, wood shavings, performance

214 The comparison of turkey and Pekin duck embryos exposed to embryonic thermal manipulation on heat shock protein 70 (HSP70) mRNA and post-hatch immune response to a lipopolysaccharide challenge. R. Shanmugasundaram* and M. Lilburn, *OARDC, Ohio State University, Wooster, OH.*

Embryonic temperature manipulation has been proposed as a means of modulating the effects of post-hatch heat stress. The objective of this experiment was to expose duck and turkey embryos to thermal manipulation to study their post-hatch immune response to a lipopolysaccharide (LPS) challenge. Commercial Pekin duck and turkey eggs were exposed to 4 treatments: SS-Control (37.5C from embryonic day [ED] 1 to 25); SS-LPS (37.5C from ED1 to 25 + LPS at D0 [hatch]); HH-LPS (38C from ED1 to 25+ LPS at D0; and SH-LPS (37.5C from ED1 to 10 and 38C from ED 11 to 25 + LPS at D0). At each time point, data was analyzed by a one-way ANOVA and Tukey post-test mean separation ($P < 0.05$). At ED10 and ED18, the shell temperature (EST) of eggs set at 38C was increased ($P < 0.05$). In both ducklings and poults, the HH treatment decreased yolk free wet embryo body weight at hatch ($P < 0.05$). At 24, 48, and 72 h post-LPS injection, the SH-LPS treatment increased BW while the HH-LPS treatment decreased BW compared with the SS-LPS treatment ($P < 0.05$). In both species, the SH-LPS and HH-LPS treatments increased plasma heat shock protein 70 (HSP70) and decreased splenic HSP70 mRNA compared with the SS-LPS treatment at all time points post-LPS injection ($P < 0.05$). At 48 and 72 h, macrophage nitric oxide (NO) production in the SH-LPS and HH-LPS treatments was lower than the SS-LPS treatments ($P < 0.05$). Ducklings and poults in the SH-LPS treatment had an increased thymocyte proliferation index compared with the SS-LPS treatment at 24, 48 and 72 h post-LPS injection ($P < 0.05$). At 24 h post-LPS injection, ducklings in the SH-LPS treatment had increased splenic IL-6 and IL-10 and reduced IFNγ mRNA abundance, while both ducklings and poults in the HH-LPS treatment had increased IFNγ and IL-6 mRNA abundance compared with the SS-LPS treatment ($P < 0.05$). At 48 h, the SH-LPS ducklings and poults had lower splenic IL-10 mRNA abundance ($P < 0.05$) while the HH-LPS treatment resulted in comparable ($P > 0.05$) splenic IL-10 mRNA abundance compared with the SS-LPS treatment. Ducklings and poults in the SH-LPS treatment had increased thymic and splenic $CD8^+/CD4^+$ ratiosat 24 post-LPS injection compared with the SS-LPS treatment ($P < 0.05$). In conclusion, embryonic thermal manipulation from ED11–25 increased the release of extracellular HSP70, decreased HSP70 splenic mRNA amounts, increased thymocyte proliferation and IL-10 while decreasing IFNγ mRNA amounts at 24 h post-LPS injection. This suggests that a small increase in incubation temperature during the later stage of incubation could potentially prime the immune system by downregulating HSP70 mRNA and increasing the extracellular release of HSP70.

Key Words: thermal manipulation, HSP70, lipopolysaccharide challenge, immune response

215 Restricting factors to local chicken backyard production system in Campeche State, Mexico. A. Mata-Estrada[1], F. González-Cerón[2], A. Pro-Martínez[1], G. Torres-Hernández[1], J. Bautista-Ortega*[3], A. Vargas-Galicia[1], C. Becerril-Pérez[4], E. Sosa-Montes[2], and J. Arreola-Enríquez[3], *[1]Colegio de Postgraduados, Campus Montecillo, Texcoco, Estado de México, Mexico, [2]Universidad Autónoma Chapingo, Texcoco, Estado de México, Mexico, [3]Colegio de Postgraduados, Campus Campeche, Champotón, Campeche, Mexico, [4]Colegio de Postgraduados, Campus Veracruz, Veracruz, Veracruz, Mexico.*

Backyard chicken production consists in raising local chickens that are adapted to the environmental conditions of the region; this activity supports the economy of rural families. Characterization of backyard chicken production systems was conducted from June to November 2017 aiming at knowing the limiting factors faced by local farmers who produce and market local chickens in Campeche, Mexico. A survey involving 260 households was conducted in 11 rural locations chosen at random, using structured questionnaires. Data were analyzed using frequency statistics (PROC FREQ) of SAS software. The results showed that the feed shortage (36.5%), diseases (flu and diarrhea; 21.9%), and predators (foxes, possums, and dogs; 13.5%) were identified as the major constraints for backyard production of local chickens, which is aggravated by the lack of technical assistant services (83.9%). Insufficient production of local chickens (46.0%) and lack of market place (42.5%) were the found to be the main obstacles for marketing of meat and eggs. Despite challenges for raising and marketing local chickens, about 89.0% of farmers would like to raise more chickens in their backyard, with the purpose of having a source of food and income for their family. Thus, results of this study suggest that extension services are necessary to help overcome the existing limiting factors of producing local chickens in backyard production system. Accordingly, it is considered necessary to create a program that fosters the raising of local poultry genetic resources in Campeche State, Mexico.

Key Words: limiting factors, backyard local chicken, Campeche State, disease, insufficient production

216 Synthetic allicin may improve survival in broilers raised at high altitude. A. Vargas-Galicia[1], R. Argüello-García[2], A. Pro-Martínez[1], F. González-Cerón[3], J. Bautista-Ortega*[4], E. Sosa-Montes[3], L. Rodriguez-Ortega[5], J. Gallegos-Sánchez[1], C. Ruiz-Feria[1], and J. Arreola-Enríquez[4], *[1]Colegio de Postgraduados, Campus Montecillo, Texcoco, Estado de México, Mexico, [2]Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional, Mexico City, Mexico, Mexico, [3]Universidad Autónoma Chapingo, Texcoco, Estado de México, Mexico, [4]Colegio de Postgraduados, Campus Campeche, Champotón, Campeche, Mexico, [5]Universidad Politécnica de Francisco I. Madero, Tepatepec, Hidalgo, Mexico.*

The aim of this study was to evaluate the effect of synthetic allicin on cumulative feed intake, BW gain and survival percentage in broilers. A total of 210 1-d-old male broiler chicks (strain: Ross 308) were randomly

assigned to one of 3 treatments: 0 mg of allicin kg^{-1} of BW (0-ALLI or control), 1 mg of allicin kg^{-1} of BW (1-ALLI) and 2.5 mg of allicin kg^{-1} of BW (2.5-ALLI); 7 replicates per treatment were used. Ascites syndrome was induced by the combined effects of raising the chickens at 2278 m of altitude (natural conditions of hypoxia) and ad libitum feeding of pelleted diet. All treatments were applied from 14 to 27 d of age and were administered by oral-esophageal route using a pediatric catheter (diameter of 1.67 mm). Treatments were administered at pH 6.5 and 5°C using distilled water as vehicle. Cumulative feed intake and BW gain, and percentage of survival birds were recorded from 14 to 42 d of age. Data were tested for deviations from a normal distribution using the Shapiro-Wilks W test and were analyzed as a one-way ANOVA. Means were separated using the Tukey test. No significant differences were found among treatments for cumulative feed intake (0-ALLI = 4230 ± 175 g $bird^{-1}$, 1-ALLI = 4413 ± 175 g $bird^{-1}$ and 2.5-ALLI = 4304 ± 175 g $bird^{-1}$) or cumulative BW gain (0-ALLI = 2612 ± 113 g $bird^{-1}$, 1-ALLI = 2725 ± 113 g $bird^{-1}$ and 2.5-ALLI = 2593 ± 113 g $bird^{-1}$). Similarly, the percentage of survival birds to ascites syndrome was not affected by treatment (0-ALLI = 68.6 ± 9.2%, 1-ALLI = 62.9 ± 9.2% and 2.5-ALLI = 75.8 ± 9.2%). In conclusion, synthetic allicin did not affect cumulative feed intake and BW gain, and percentage of survival variables in broilers. Further studies are needed to find the optimal dose and administration period of allicin that may improve the variables studied.

Key Words: synthetic allicin, survival percentage, ascites syndrome, high altitude, broiler

Rapid Communications in Research: A Showcase for the Future

217 Addition of the microencapsulated feed additive AviPlusP to a broiler's diet affects oxidative burst and nitric oxide production in peripheral blood leukocytes. C. Swaggerty*[1], H. He[2], K. Genovese[2], M. Kogut[2], A. Piva[3], and E. Grilli[3], *[1]USDA, College Station, TX, [2]USDA/ARS, College Station, TX, [3]Vetagro S.p.A., Emilia, Reggio, Italy.*

AviPlusP is a microencapsulated feed additive that increases growth rate and feed efficiency in chickens. The objectives of this study were to determine if the addition of AviPlusP to a broiler diet has any affect on the immunological functions of peripheral blood leukocytes (PBL). Day-old chicks (n = 1200) were randomly assigned to one of 3 groups: Control diet, 0 g/ton AviPlusP or a diet supplemented with 300 or 500 g/ton AviPlusP. At 4 and 14-d-of-age, peripheral blood was collected (100/group/day) and heterophils and monocytes isolated (n = 2/day). Heterophils from birds from each group were assayed for the ability to generate an oxidative burst response while nitric oxide (NO) production was measured in monocytes. Statistical analysis performed using Student's *t*-test comparing each supplemented diet independently back to the control. The data for d 4 and 14 (n = 2/day) were analyzed separately ($P \leq 0.05$). At 4 d, heterophils isolated from birds fed 500 g/ton AviPlusP had higher ($P \leq 0.05$) oxidative burst response following stimulation than those isolated from birds on a control diet. Similarly, NO production was significantly ($P \leq 0.05$) higher in monocytes isolated from birds fed the 300 and 500 g/ton AviPlusP diets following stimulation. In both functional assays, the baseline levels were comparable in heterophils and monocytes from birds fed each of the diets. After 14 d, the heterophil-mediated oxidative burst response was lower ($P \leq 0.05$) in birds fed 500 g/ton AviPlusP compared with those isolated from birds fed the control diet while heterophils isolated from birds fed 300 g/ton AviPlusP were unchanged. Baseline data indicate non-stimulated heterophils from birds fed 300 g/ton AviPlusP were higher than non-stimulated heterophils from birds on the control diet. Production of NO by monocytes after 14 d was higher ($P \leq 0.05$) in birds isolated from birds fed 300 g/ton AviPlusP while lower ($P \leq 0.05$) in monocytes isolated from birds fed 500 g/ton AviPlusP compared with cells isolated from control fed birds. Non-stimulated levels were similar in monocytes isolated from birds given each diet. In conclusion, after 4 d of supplementing with AviPlusP, PBL isolated from chicks had enhanced immunological functions of oxidative burst and NO production compared with PBL from non-supplemented birds. By 14 d, the heterophil response was dampened suggesting and anti-inflammatory effect and the monocyte function was dependent on the dose. Collectively, the data suggest AviPlusP has immuno-modulatory affects on the functionally inefficient and undeveloped immune system of young chicks.

Key Words: feed additive, microencapsulated, nitric oxide, oxidative burst, peripheral blood leukocyte

218 Effects of a dry hydrogen peroxide (DHP) sanitation system used in an egg cooler on hatchability and chick quality. B. Jordan*[1], E. Melo[2], J. McElreath[1], R. Stephens[3], L. Lara[2], N. Cox[4], and J. Wilson[1], *[1]The University of Georgia, Athens, GA, [2]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [3]Synexis Biodefense, Kansas City, MO, [5]USDA/ARS, Athens, GA.*

In commercial poultry production, hatcheries are a source of continual contamination. A sanitation method that could continually clean and disinfect a hatchery in support of daily cleaning procedures would be valuable and, for this reason, a novel, commercially available, gaseous dry hydrogen peroxide (DHP) system has been introduced to continually combat microbes in the air and on surfaces in hatcheries. Preliminary studies have shown that this sytem can indeed reduce the microbial population in hatcheries and on the surface of eggs, however the effects of this new system on hatchability and chick quality need to be evaluated. A total of 3,960 fertile eggs were collected from an approximately 40 week old Ross 308 broiler breeder flock maintained at the Poultry Science research farm and distributed in 2 treatments: control (no disinfection) and treated. For the treated group, one DHP air sanitizer was placed inside an egg cooler at the Poultry Science research farm and 2 other machines were placed in the common area outside. Both areas were treated for 7 d before placement of eggs, and then eggs were collected and placed inside the cooler over a 4 d period. Eggs were stored for 3 d after the last collection before placement in the incubator. During pretreatment and egg storage, DHP levels were measured inside the cooler and air samples were taken to evaluate environmental microbial load. After storage, eggs were placed into a single stage Natureform incubator. For the control group, all DHP machines were removed from the cooler and external room 5 d before placing the eggs and the egg cooler was cleaned. During the treated phase of the trial, a daily increasing level of DHP was measured in the egg cooler, with an average level of 12 ppb, and a concomitant reduction in microbial load from air samples was seen, confirming that the room was treated and the system was destroying microbes as expected. A 1.1% increase in hatch and a 2.5% increase in hatch of fertile were seen for the treated group, as well as a 1.7% decrease in early dead and a 1% decrease in late dead embryos. In total, this data demonstrates the positive effect that reducing microbial loads on hatching eggs using the DHP system can have on hatchability.

Key Words: dry hydrogen peroxide, hatchability, microbe

219 Reductions of dietary crude protein in Cobb 500 broilers fed diets balanced in essential amino acids and nitrogen. M. Kidd*[1], R. Brister[2], P. Tillman[3], D. Aldridge[1], L. Butler[1], and S. Liu[1], *[1]University of Arkansas, Fayetteville, AR, [2]Tyson Foods, Springdale, AR, [3]PTNS LLC, Buford, GA.*

Protein efficiency worsens in broilers fed excess amino acid-high CP diets, whereas feed efficiency worsens in broilers fed limited amino acid-low CP diets. Two experiments (Exp.) were conducted to assess the sensitivity of protein supply and amino acid balance (balance in Exp. 1 refers to essential amino acids; and in Exp. 2 refers to essential amino acids and N) in Cobb 500 male broilers. In Exp. 1, 14 to 35 d broilers received diets from 16.0 to 20.0% CP in 0.8% increments (6 CP levels replicated 5 times with 12 birds per replicate). In Exp. 2, 14 to 35 d broilers received the 16.0 and 20.0% CP diets balanced in essential amino acids, and the 16% CP diet supplemented with L-Leu, L-Gln, and L-Gly to achieve 4% added CP (5 replicates of 12 birds each). Broilers were reared in floor pen settings with built-up litter. In Exp. 1, linear live performance improvements ($P < 0.05$), but not quadratic, in response to increased CP were observed. For processing yields, as CP increased carcass yield increased linearly ($P < 0.05$), thigh and fat yield decreased linearly ($P < 0.05$), and breast yield had a quadratic increase ($P < 0.05$). In Exp. 2, although protein efficiency ($P \leq 0.01$) was improved in birds fed the low CP diet supplemented with nonessential amino acids, BW gain and feed conversion were negatively impacted ($P < 0.05$) in birds fed the low CP diet with or without the nonessential amino acid addition. Front half yields (breast) were improved ($P < 0.05$) in birds fed the 20% CP diet, but back half yields (thighs and drumsticks) were improved (*P*

< 0.05) in birds fed the low CP diets. Treatments did not alter ($P > 0.05$) plasma amino acids. Results revealed Cobb 500 broiler sensitivity to amino acid supply as well as potential to reduce diet CP.

Key Words: crude protein, broiler, essential amino acid, nonessential amino acid, nitrogen

220 Effects of light intensity and dual light choice treatments on growth performance of male broilers. K. Christensen*[1], S. Kang[2], D. Aldridge[2], and W. Kuenzel[2], *[1]Tyson Foods, Inc., Springdale, AR, [2]University of Arkansas, Fayetteville, AR.*

Light intensity is an area of interest for maximizing welfare of commercial broilers. Previous research indicates that given a choice, birds show a preference for greater light intensity during the photoperiod and broilers may prefer more intense light at the feeders and less intense light areas to rest between meals. A study was conducted to investigate the possible beneficial effect of light intensity and dual light choice on the performance parameters of male broilers. Day old broilers were housed in 2 commercial broiler houses, and placed in 20 pens (10 pens/house, 11 birds/pen, 8 pens/trt). All of the pens were supplied with 23:1 h of light at 40 lx (lx) during the first 14 d of age before the onset of light treatments. On Day 15, 4 treatments (2, 10, 20 and 100 lx) and one choice treatment (2/20 lx) were initiated. Performance parameters of body weight (BW) and feed conversion ratio (FCR) were determined for each light intensity and dual light choice at Day 15 and 41. Differences in treatment groups were analyzed by using one-way ANOVA followed by mean separation test using Tukey's HSD test (Significance $P < 0.05$; JMP 11). Plasma corticosterone (CORT) levels measured at Day 16, and 17 showed that CORT levels of 100 lx treated group and choice group were lower than 2, 10, and 20 lx treated birds ($P < 0.05$). At Day 15, body weights were similar across all treatments including the dual light choice treatment. Similarly, FCR at Day 15 were not significantly different suggesting that the light treatments and environment were similar before the onset of the different lighting treatments. At Day 41, BW of birds raised under 100 lx were lower in BW (2.873kg) than those of birds raised under 10 and 20 lx (3.035 and 3.009 kg) but not different than BW of 2 lx or the dual light choice trt birds. The FCR on Day 41 was not different among treatments but numerically, 2 lx and the dual light choice trts were the lowest. Taken together, results suggest that birds with dual light choice treatment (2/20 lx) appear to have similar growth performance parameters with low light intensity treated birds (2, 10, and 20 lx) and have better growth performance parameters (lower FCR and higher BW) than birds treated with bright light intensity (100 lx). This trial suggests that providing commercial broilers with a choice in light intensity may simulate the natural preference of eating behavior in a higher light intensity and resting in lower light simulating natural cover with better growth performance.

Key Words: light intensity, choice preferencee, growth perfomance, poultry welfare

221 The Animal Food and Nutrition Consortium: A collaborative model to fund pre-competitive research and career development. P. Ferket*, *NC State University, Raleigh, NC.*

The Animal Food & Nutrition Consortium (AFNC) is a new industry-member supported consortium with the mission to promote innovation and advances in the nutrition, metabolism and gastrointestinal health of production and companion animals. The research focus is on precompetitive research topics, driven by industry needs. The AFNC provides a framework to bring industry partners together with an interdisciplinary team of over 200 food animal faculty distributed across at least 3 NC State University departments in the College of Agriculture and Life Sciences (Animal Science, Prestage Department of Poultry Science, Applied Ecology), the College of Veterinary Medicine, and the College of Sciences. The consortium sponsors pre-competitive research and solutions that encompass the food and nutrition of production and companion animals including, but not limited to: the potential value of feed ingredients and additives; digestive physiology; gut health and the enteric microbiome; impact on environmental sustainability and climate change, efficiency of nutrient utilization and minimization of environmental emissions; animal food quality and safety; integration of nutrition and animal welfare and well-being; big data analytics; and other emerging issues relevant to livestock, aquaculture and companion animals. Benefits accrue to all AFNC partners in the form of resources for faculty research, leveraged research funding for industry, and developing the next generation of industry employees and policy makers. Ultimately, the consumer wins too, as research results are expected to improve the value and quality of food animal products (meat, milk, and eggs) and companion animal foods, improve environmental sustainability by lowering nutrient emissions, and enhance animal health and welfare. The AFNC provides defined engagement rules for members from competing companies with an opportunity to leverage their investments through one of 3 membership fee tiers: $50,000 for Full Membership with the privilege of 2 votes at Advisory Board meetings, $25,000 for Associate Membership with one vote, and $10,000 for Non-profit Membership (no voting rights). Any intellectual property generated from the consortium will be available for non-exclusive license to Full or Associate Members in good standing. Future extension and education components of the AFNC include Industry Workshops, Academic Symposia, Graduate student fellowships, Undergraduate scholarships, and the AFNC Summer Institute.

Key Words: industry consortium, funding model, industry-academic collaboration, research, career development

222 Delayed access to feed impacts developmental gene expression in young broiler breast muscle. J. Payne*[1], M. Proszkowiec-Weglarz[2], and L. Ellestad[1], *[1]Universtiy of Georgia, Athens, GA, [2]USDA, ARS, NEA, Beltsville, MD.*

In commercial settings, between hatch and placement of chicks, there is a variable length of time where birds do not have access to feed. When this period is prolonged by differences in time of hatch and/or transportation, birds can be left without feed for up to 48h. The objective of this study was to investigate effects of a prolonged delay in access to feed on gene expression in breast muscle. Chicks were given access to feed within 3h of hatch (fed) or were delayed access for 48h (delayed), with 6 replicate pens per group. Birds were weighed every 2 d from hatch until post-hatch day (D)14, and tissue was collected at hatch, 4 h post-hatch, D1 (24h), D2 (48h), D4, and D8. Total RNA was isolated from breast muscle collected from 4 males in each treatment at each time point and analyzed by quantitative PCR for genes regulating growth, development, and metabolism. Data were analyzed by 2-way ANOVA, and differences between groups were determined using Tukey's multiple comparison model. After D6, birds from the delayed group were significantly lighter then fed birds through the end of the study ($P < 0.01$; n = 6). In the delayed group on D2 the following differences in mRNA expression as compared with fed birds were observed (n = 4): 28-fold increase in FBXO32 ($P < 0.05$), 7-fold decrease in MYOG ($P < 0.05$), 9-fold decrease in MSTN ($P < 0.05$) and 2-fold increase in INSR ($P = 0.057$). An apparent reduction in MSTN mRNA in the delayed group was also observed on D4 ($P = 0.072$), with no differences between treatments at d

8. The altered developmental expression of INSR, FBXO32 and MYOG in the delayed group may indicate lower blood glucose, increased protein degradation, and decreased muscle growth due to delayed access to feed, respectively. Reports from knockout models suggest that MSTN is a negative regulator of muscle growth; however, the expression pattern in birds deprived of feed suggest that it may play a different role during early post-hatch development when birds are nutrient deprived. A 48h delay in feeding is sufficient to alter gene expression regulating growth, development, and metabolism in breast muscle, which may contribute to the reduced body weight observed. The lack of differences in gene expression during the first 24h of feed restriction indicate that placement of chicks within this time frame may mitigate detrimental effects on gene expression that contribute to a reduction of body weight gain and alterations in breast muscle development.

Key Words: delayed feed, hatch, breast muscle, development, gene expression

223 Determination of microbial populations in ceca of chickens by microbiome and bacterial culture techniques: A comparison. K. Wilson[1], W. Briggs[1], A. Duff[1], K. Chasser[1], X. Sun[2], and L. Bielke*[1], *[1]Ohio State University, Wooster, OH, [2]University of Arkansas, Fayetteville, AR.*

The use of next generation sequencing (NGS) technology to characterize microbial communities have become the standard when studying microbial populations of the gastrointestinal tract, mainly due to the ability to identify microbiota that cannot be observed via culture-based methods. However, culture-based techniques are acceptable for identification of some bacterial groups, though it may grossly underestimate microbial communities, and limited research directly comparing NGS results to cfu counts exists in literature. The objective of this study was to compare total *Enterobacteriaceae* and lactic acid bacteria (LAB) recovery by culture techniques to operational taxonomic unit (OTU) generated from Illumina MiSeq sequencing of matched chick cecal samples at 3 and 10 d post hatch. The relationship between cfu and OTU may not be relatable at d3 where total recovered *Enterobacteriaceae* represented 64.8% of the total relative cfu/g, while the remaining 35.2% belonged to LAB. This result was contradicted at the OTU level, where *Enterobacteriaceae* represented 22.2% of the total relative OTU counts and the remaining 77.8% belonged to LAB. For *Enterobacteriaceae* comparison of cfu and OTU counts, the Pearson correlation value, tested by simple linear regression model, was very low at −0.0562 ($P = 0.76$), whereas LAB cfu and OTU comparison had a correlation of 0.0025 ($P = 0.94$). By d10 the relative abundance of cfu and OTU values of *Enterobacteriaceae* were numerically similar with 8.1% and 4.6%, respectively, and LAB counts of 91.9% and 95.4%, respectively. Colony-forming units may be appropriate to identify a few families or orders of culturable bacteria that change in relation to a treatment or product, but NGS appears to be favorable for total population changes. Differences between cfu determination and NGS may be attributed to culturability of bacteria and should be considered when completing analysis of microbial populations.

Key Words: microbiome, bacteriology, OTU, colony-forming unit

224 Assessment of stabilized hydrogen peroxide as an antimicrobial agent for use in reducing *Campylobacter* prevalence and levels on chicken broiler wings. D. Bourassa*[1], C. Harris[2], N. Bartenfeld[3], and R. Buhr[3], *[1]Auburn University, Auburn, AL, [2]University of Georgia, Athens, GA, [3]USDA-ARS, Athens, GA.*

The USDA Food Safety and Inspection Service requires samples of raw broiler parts for performance standard verification for the detection of *Campylobacter*. Poultry processors must maintain process controls with *Campylobacter* prevalence levels below 7.7%. Establishments utilize antimicrobial processing aids on broiler parts to aid in achieving the standards. In this study, a stabilized hydrogen peroxide (SHP) product was assessed for use as an antimicrobial processing aid. In 2 trials, wing segments with skin (drummettes and flats) were inoculated with approximately 10^{5-6} cells of a marker strain of *Campylobacter coli* and allowed 1–2 h to attach before treatment. In Trial 1, treatments were SHP at 15,000 ppm and water applied by either a 1 min dip (2 segments in 100 mL) or spray (2 segments hand sprayed with 50 mL). Following treatments, samples were either analyzed the same day for *Campylobacter* or after refrigeration for 24 h. In Trial 2, treatment groups were SHP at 15,000 or 30,000 ppm and water applied by either a 1 min dip or spray as in Trial 1 and samples were analyzed the same day. Rinsates from each part were analyzed for direct counts and prevalence of the marker *Campylobacter*. Count and prevalence data were statistically analyzed by GLM with means separated by Tukey's HSD test or Fisher's Exact test with main effects of antimicrobial, application method, and time in Trial 1 and antimicrobial and application method in Trial 2. In Trial 1 for both counts and prevalence, only the antimicrobial main effect of was significant with no interactions. Treating wing segments with SHP at 15,000 ppm significantly decreased the levels of *Campylobacter* (1.10 $\log_{10}$ cfu/mL) compared with the water (1.64 $\log_{10}$ cfu/mL). Prevalence was also significantly decreased from 77% (water) to 53% (SHP) positive. In Trial 2, both main effects of antimicrobial and application method were significant for counts with no interactions. Water had significantly higher *Campylobacter* counts than SHP at both 15,000 and 30,000 ppm (0.92 vs. 0.40 or 0.28 $\log_{10}$ cfu/mL, respectively). The dip application method had significantly lower *Campylobacter* counts than the spray application method (0.40 vs. 0.64 $\log_{10}$ cfu/mL). *Campylobacter* prevalence was significantly lower for wing segments treated with 30,000 ppm (70%) than wings treated with 15,000 ppm SHP (93%). These results indicate that while there was no carry-over effect of the SHP (same day vs. 24 h), SHP was able to significantly decrease levels of *Campylobacter* on broiler chicken wing segments compared with water and dipping parts was more effective than spraying. SHP should be further investigated as a potential antimicrobial processing aid for use on poultry parts.

Key Words: *Campylobacter*, hydrogen peroxide, broiler, wing

225 Consequences of genetic selection on hormonal systems regulating skeletal health in broiler chickens. L. Ellestad*, J. Payne, and G. Boothby, *University of Georgia, Athens, GA.*

Dramatic improvements in broiler growth performance resulting from genetic selection are associated with negative consequences, such as impaired immune function, reproductive inefficiency, and musculoskeletal disease. Vitamin D_3 (cholecalciferol) plays an important role in calcium and phosphorus homeostasis and is essential for bone strength. Several studies have demonstrated that feeding alternative metabolites of cholecalciferol, particularly more biologically active forms [25(OH)D_3 or 1,25$(OH)_2D_3$], can improve performance and bone health. However, there remains a lack of data relating to regulation of vitamin D conversion into these biologically active forms and subsequent hormonal regulation of calcium and phosphorus metabolism in broilers. The objective of this study was to determine if genetic selection has impacted conversion of exogenous vitamin D_3 to its metabolites in liver and kidney or affected levels of receptors for hormones that are critical in regulating calcium and phosphorus metabolism. On 21

d post-hatch, liver and kidney samples were collected from 8 modern broilers (Cobb 500) and 8 Athens Canadian Random Bred (ACRB) birds that represent a population from the mid-1950s (n = 8). Total RNA was isolated, reverse transcribed, and mRNA levels for enzymes involved in conversion and activity of vitamin D were measured [vitamin D 25-hydroxylase (CYP2R1), vitamin D 24-hydroxylase (CYPR24A1), vitamin D receptor (VDR), and retinoid-X-receptors α (NR2B1) and gamma (NR2B3)]. Levels of mRNA for hormone receptors involved in calcium and phosphorus homeostasis were also measured [parathyroid hormone receptors 1 and 3 (PTH1R and PTH3R), and calcitonin receptor (CALCR)]. Data were analyzed by ANOVA followed by the test of least significant difference. Levels of mRNA for CYP2R1, the enzyme responsible for the first activation step converting vitamin D_3 to $25(OH)D_3$, were higher in the liver of ACRB birds, while mRNA levels of CYP24A1, the enzyme responsible for deactivating $1,25(OH)_2D_3$ by converting it to $1,24,25(OH)_3D_3$, were higher in Cobb 500 ($P < 0.05$). Expression of mRNA for NR2B3, a transcriptional partner of VDR, was higher in ACRB liver than Cobb liver, as was expression of PTH1R mRNA ($P < 0.05$). It appears that modern broilers have a reduced ability to activate dietary vitamin D, as well as increased de-activation of the hormonal form of the vitamin responsible for its biological action. Parathyroid hormone sensitivity may also be reduced in the liver of modern broilers. These results indicate that altered vitamin D metabolism and related hormonal signaling have been compromised by genetic selection and may contribute to the skeletal weakness observed in modern broiler chickens.

Key Words: bone health, vitamin D, calcium, phosphorus, metabolism

226 A precision intubation intake bioassay to re-evaluate vitamin D_3 requirements for growing broiler chickens. H. Leyva-Jimenez, M. Khan, K. Gardner, R. Abdaljaleel, Y. Al-Jumaa, A. Alsadwi, and C. Bailey*, *Texas A&M University System, College Station, TX.*

Cholecalciferol or vitamin D_3 (D_3) is added to all poultry diets for its potent antirachitic activity. It is likely that nutrient requirements of meat type chickens have changed since the last revision of the NRC in 1994 due to intensive genetic selection, improvements in flock management, feed technology and vaccination programs that have led to the most efficient poultry production in history. Moreover, literature referenced in the 1994 NRC used to establish the D_3 requirement of young growing meat-type chickens date as early as 1963. Concern among researchers and nutritionist regarding the need of re-evaluating nutrient requirements that more accurately reflect current feeding strategies and the ability of modern poultry strains to respond to dietary nutrients has increased over the last few years. Precision nutrition has been adopted worldwide as a strategy to reduce feed costs which can be as high as 70% of the total production cost. New methodologies may be appropriate that allow nutritionist to precisely estimate nutrient requirements of current poultry strains for optimal performance. For example, modern commercial diets often utilize vitamin D_3 concentrations up to 16x the 1994 minimal requirement of 200 IU D_3 /kg of the diet as beneficial effects, other than preventing classical deficiency, have been reported in more recent literature. Vitamin requirements have traditionally been determined by adding graded concentrations of the vitamin in question to a deficient diet and using broken-line regression to assess the response variables. Mixing error, selective feeding, and other sources of error including nutrient assay of the test diets are inherently problematic with this approach. Our approach utilizes a highly purified pharmaceutical grade cholecalciferol standard (99.8%) purchased from Sigma-Aldrich Chemical Company which was dissolved in corn oil to create 8 graded D_3 treatments representing from 0 to 3200 IU D_3/kg feed which were orally gavaged daily to individual chicks directly into the crop based on feed intake. Cholecalciferol intake per kg of diet was then adjusted based on daily feed intake per pen of birds. Using this methodology, the linear broken-line regression for tibia bone ash suggests a minimum D_3 requirement of 491 IU/kg of feed, whereas the quadratic broken-line regression suggests a minimum D_3 requirement of 499 IU/kg of feed.

Key Words: vitamin D, cholecalciferol, broiler, requirement, bioassay

227 Factors influencing the severity of blackhead outbreaks in turkeys. R. Beckstead*[1], J. Payne[2], K. Cupo[1], C. Sigmon[1], and E. Chadwick[1], *[1]North Carolina State University, Raleigh, NC, [2]Universtiy of Georgia, Athens, GA.*

Histomonas meleagridis is a protozoan parasite that infects a wide range of gallinaceous birds causing blackhead disease. Transmission between infected and uninfected turkeys is generally below 10%, however up to 100% mortality has been recorded. Little is known about factors that cause the variation in transmission. We hypothesized that diet, gut health and the virulence of the parasite alter transmission levels. A trial was performed to determine if changes to the diet or irritation of the gut could alter direct and lateral transmission rates. Turkeys were fed: a standard diet (diet 1), diet 1 + MgCl2, diet 1 +MgSO4, diet 1 + coarse corn or diet 1 + dextran-SO4 and a second diet (2) formulated with excess sodium and an amino acid imbalance. Diet 1 + MgCl2 or MgSO4 and diet 2 were formulated to increase fecal moisture. Coarse corn was added to stimulated reverse peristalsis. Dextran-SO4 was included to induce enteric inflammation. The infection rate for poults directly inoculated with *H. meleagridis* was: 74% diet 1, 83% diet 1 + MgCl2, 76% diet 1 + MgSO4, 94% diet 1 + dextran-SO4, 83% diet 1 + coarse corn and 94% diet 2. Diet 1 and diet 1 + MgSO4 rates of direct infection were statistically different from diet 1 + dextran-SO4 and diet 2 ($P \leq 0.05$). The rate of lateral transmission was significantly different for diet 2 compared with all other diets. To determine if the virulence of the parasite plays a role in the disease outcome, 3 *H. meleagridis* field strains (BF, ZM and MNC) and 2 BF sub-isolates (BM1 and BM2) were tested in turkeys for variation in pathogenicity. Turkeys infected with BF, ZM and MNC strains differed significantly from the uninfected birds with only the ZM and MNC strains differing from each other in the liver lesion scores ($P \leq 0.05$). BM1 differed significantly from the BF parental strain with higher cecal scores. BM2 differed significantly from the BF parental strain with increased body weight gain. Field strains associated with high mortality exhibited similar pathogenicity. Subpopulations within the BF parental strain showed variation in pathogenicity, suggesting that low pathogenic strains exist in the field and may explain some of the variation seen in blackhead outbreaks. The data presented here suggests that variations in mortality associated with field outbreaks can be associated with dietary formulations, gut health and virulence of the parasite.

Key Words: *Histomonas meleagridis*, turkey, blackhead, virulence, gut health

228 Influence of basal diet type on regression-based metabolizable energy values of dextrose determined using index and total excreta collection methods. S. West and S. Rochell*, *University of Arkansas, Fayetteville, AR.*

The metabolizable energy (ME) value of dextrose has been well-characterized in classic literature, and as such, it is often included as a component of reference diets used in ME assays. Furthermore, due to its

purified and consistent composition, dextrose serves as a good ingredient to evaluate when comparing different methodological approaches to determine the ME value of feed ingredients. Thus, an experiment was conducted to evaluate the effect of basal diet type on the nitrogen-corrected ME (ME_n) value of dextrose determined in broiler chicks using the regression method based on both index and total excreta collection (TC) procedures. Two basal diet types included a semi-purified (SP) basal based on corn, casein, and dextrose and a more practical basal based on corn and soybean meal (CSBM). The dextrose was included at 0, 22.5 and 45% at the expense of all energy-providing ingredients in both SP and CSBM diets. Titanium dioxide (TiO_2) was added at 0.5% to all experimental diets as an indigestible marker for determination of ME_n by the index method. Three-hundred and 84 male Cobb broiler chicks were randomly distributed among 48 battery cages (8 birds/cage) and fed a common starter diet for 14 d. At 14 d post-hatch, 8 replicate cages of chicks were provided 1 of 6 experimental diets until 21 d post-hatch. Total feed intake and excreta output were measured over 48 h from 19 to 21 d. All diets and representative excreta samples from each cage were analyzed for dry matter, nitrogen, gross energy, and TiO_2 concentrations. The ME_n of dextrose was determined by difference for each test diet using both index and TC procedures, and the dextrose-associated caloric intake was regressed against the amount of dextrose intake to generate linear regression equations with slopes corresponding to the ME_n value of dextrose within each basal type. Based on overlapping 95% confidence intervals of the resulting slopes, ME_n values of dextrose determined using the TC method were not different between the SP (3,504 kcal/kg) and CSBM (3,410 kcal/kg) basal diet types. Using the index method, there was a relatively larger difference between the ME_n values of dextrose determined in SP basal diets (3,568 kcal/kg) and in CSBM basal diets (3,802 kcal/kg), but these values also had overlapping 95% confidence intervals. These results indicate that both the index method using TiO_2 as an indigestible marker and TC procedures may be reliably used to characterize the ME_n content of feed ingredients. Furthermore, it is possible to obtain similar estimates of ingredient ME_n in CSBM and SP diets, potentially allowing for simultaneous determination of ME_n in amino acid digestibility assays that utilize SP diets.

Key Words: dextrose, semi-purified diet, basal type, metabolizable energy, broiler

229 Impact of egg holding temperatures on the recovery of inoculated *Salmonella* from eggshells and stainless steel coupons. R. Buhr*[1], D. Bourassa[2], and K. Wilson[3], *[1]USDA ARS, Athens, GA, [2]Auburn University, Auburn, AL, [3]Ohio State University, Columbus, OH.*

This experiment was conducted to determine the impact of egg holding temperature on the ability to recover *Salmonella* from eggshells after 24 h. *Salmonella enterica* Enteritidis (nalidixic acid resistant marker strain) inoculated eggshells and stainless steel coupons (SSC, 14 mm diameter) were held at 24, 19, or 7°C for 24 h before sampling. These 3 holding temperatures represent environments that eggs could be kept at after lay; room temperature, egg cooler (hatching eggs), or refrigerated (table eggs). Inoculated SSC were used to assess the survivability of the inoculated *Salmonella* on a non-eggshell surface. Inoculated eggs and SSC were also placed in a freezer at −15°C and served as an additional treatment group. In the first trial, eggs were challenged with *Salmonella* inoculum at either 10^4 or 10^5 cfu/10 µL and in the second trial eggs and SSC were challenged with *Salmonella* inoculum at 10^5 cfu/10 µL. After holding, the inoculated eggs were cracked on a clean aluminum foil surface and the internal contents were discarded. Eggshells were crushed in a gloved hand and put into a 50 mL centrifuge tube. Each sample received 30 mL of 1% buffered peptone water and was incubated at 37°C for 18 to 24 h. Two loops (20 µL) of eggshell or SSC rinsates were streaked onto Brilliant Green Sulfa agar containing 100 µg/mL nalidixic acid. Plates were incubated for 24 h at 37°C, after which the plates were recorded as positive or negative. Eggshells inoculated at 10^4 or 10^5 cfu and sampled after holding at room temperature (24°C) for 1 h had recovery from 80 to 97% *Salmonella* positive, indicating a successful challenge. In the first trial inoculated eggs held for 24 h at 24, 19, or 7°C had low recovery of *Salmonella* from 0 to 27% (0 to 1/15 for inoculum at 10^4 cfu and 0 to 4/15 for inoculum at 10^5 cfu). In the second trial eggs and SSC inoculated at 10^5 cfu and sampled after 1 h had *Salmonella* recovery of 97% (29/30) for eggshells compared with 93% (14/15) for SSC. However, *Salmonella* recovery after 24 h significantly differed ($P < 0.05$) between samples held at −15°C (0% for eggshells vs. 93% for SSC); samples held at 7°C (57% for eggshells vs. 100% for SSC); samples held at 19°C (0% for eggshells and 87% for SSC); and for those samples held at 24°C (20% for eggshells and 100% for SSC). Overall for the second trial after holding for 24 h at 24, 19, or 7°C, *Salmonella* was recovered from only 19% (23/120) of the eggshells but from 95% (57/60) of the SSC. These results indicate that sampling of eggshells for *Salmonella* 24 h after inoculation may not accurately represent pathogen levels present at lay. Additionally, the eggshell appears to have an antimicrobial effect against *Salmonella* during this 24-h time period

Key Words: eggshell, *Salmonella*, holding temperature

230 Modernizing poultry processing through Big Data analytics and blockchain. A. Morey*, *Auburn University, Auburn, AL.*

Poultry processors are constantly faced with the pressure to maintain a high throughput operation while satisfying the quality parameters and stringent USDA food safety regulations in a dynamic environment. Processors often have to make fast decisions and may not have the time to analyze all the incoming data and assess the trends to fully comprehend the situation. A wrong decision may incur significant economic losses to a company. Hence, the processors must be equipped with modern Big Data analytics tools which are capable of processing the high volume, high velocity and high variety of data shared on blockchain ledgers. Poultry companies collect data at several different locations from breeders, hatcheries, farms (e.g., climate conditions, growth rate, feed quality and safety, ammonia levels, litter quality) as well as at several locations in the processing plant. Big Data analytics allows the companies to study the known correlations between the collected data as well as the hidden correlations which may affect food safety, quality and throughput of the processing plant. For example, data on pre-harvest climate conditions (e.g., rainfall, temperature, relative humidity) combined with litter management practices, ammonia levels in the house among others can potentially predict prevalence levels of *Salmonella* in the flock and alert the processors on the incoming broiler flock. Such information can be used to adjust the antimicrobial concentrations, introduce an additional hurdle or take additional steps to improve food safety. However, rapid decision making requires real-time data collection which can be conducted using sensors retrofitted on processing equipment, trucks, farms and other areas of importance. The data being collected must also be distributed to the concerned parties in a trusted manner such that the data does not get tampered. Blockchain ledgers can be used to share the data being collected and distribute it securely preventing data fraud. Blockchain can help processors in improving traceability of the product, rapidly identify the source of the product involved in the recall within seconds as well as identify the error in the system. Big Data and

blockchain can modernize poultry processing operations, however these concepts are very novel to several companies with unproven benefits to the processors. There is a need to conduct systematic research to demonstrate the significant of these technologies to the poultry processors.

Key Words: Big Data, poultry, blockchain, poultry processing

231 Evaluation of a mechanical cervical dislocation tool for growing and mature turkeys. T. Widowski*[1], C. Woolcott[1], P. Turner[1], K. Schwean-Lardner[2], L. Caston[1], and S. Torrey[1], *[1]University of Guelph, Guelph, ON, Canada, [2]University of Saskatchewan, Saskatoon, SK, Canada.*

On-farm killing of compromised poultry is an important animal welfare issue, and is particularly difficult to perform on turkeys. Manual application of cervical dislocation (CD) is limited by size of the bird and strength of the stockperson, and therefore, mechanical tools have been developed for cervical dislocation of larger birds (MCD). Criteria for acceptability of cervical dislocation are rapid loss of consciousness (sensibility) and luxation of cervical vertebrae (ideally at C0-C1) without primary crushing (AVMA, 2013). The objective of this study was to evaluate the efficacy of a MCD tool (Koechner Euthanizing Device, Koechner Mfg. Co., Tipton, MO) when used on cull turkeys at 8 (5.2 kg, KED-C, n = 7), 15 (14.5 kg, KED-C, n = 8) and 18 (19.4 kg, KED-T, n = 10) wks of age (woa). Device model used (KED-C or KED-T) was based on manufacturer recommendations for body weight. Six additional 8-woa turkeys were killed by CD. Killing methods were performed by operators experienced with CD and mechanical devices. Presence or absence of jaw tone (JT), nictitating membrane reflex (NM), pupillary light (PL) reflex and gasping was recorded every 15 s following application. Estimated time of cardiac arrest (CA) was determined by auscultation. A sample of survey radiographs was used to determine site of dislocation and presence of vertebral fractures. Separate one-way ANOVA (GLM, SAS 9.4) tested effects of method (CD vs MCD) at 8 woa, and effects of age (for MCD only). At 8 woa, loss of JT, NM, and PL took longer (all $P < 0.005$) for MCD (156.0 ± 10.6, 175.7 ± 13.5, 175.7 ± 15.4s, respectively) than CD (65.0 ± 9.7, 67.5 ± 14.6, 95.0 ± 16.6s). CA was not different ($P = 0.44$), averaging 187.5 s. Latencies to loss of JT, NM, and PL following MCD differed with age (all $P < 0.05$) averaging 277.5 ± 20.3, 283.1 ± 23.1, 283.1 ± 21.7s at 15 woa and 205.5 ± 18.2, 239.4 ± 13.3, 239.6 ± 12.7s at 18 woa. CA was detected at 195.0 ± 13.22, 296.2 ± 12.4, 267.0 ± 7.2s, at 8, 15, and 18 woa, respectively ($P < 0.001$). Gasping was observed in 2 of 6 birds killed by CD and all birds killed by MCD. At 15 woa, luxation occurred between C0-C1 in 4 of 8, C1-C2 in 5 of 8, and C2-C3 in 1 of 8 birds (more than 1 site in 2 birds). Fractures were observed in 5 of 8 birds and included transverse and comminuted fractures and fragmentation. Using JT as an indicator, loss of sensibility occurred at around 2.5 min at 8 woa, 4.5 min at 15 woa and 3.5 min at 18 woa when birds were killed with the KED. MCD with KED caused luxation, but fractures were also observed in over half of the 15-woa birds. It is debatable as to whether this device meets the criteria for humane killing.

Key Words: on-farm killing, mechanical cervical dislocation, turkey, loss of sensibility, vertebral fractures

232 Wooden breast myopathies start with changes in calcium signaling and ends with cancer. K. Livingston*, M. Livingston, and C. Ashwell, *North Carolina State University, Raleigh, NC.*

Wooden breast (WB) is a muscle myopathy that started plaguing the poultry industry in recent years. It typically occurs in the fastest growing, high-yield male broilers. The *pectoralis major* is the primary affected tissue and is palpably hard to the touch. The meat has an extremely dry and mealy feel to consumers, which is undesirable. Moreover, when tissue is evaluated histologically, there appears to be an infiltration of white blood cells. Previous studies have evaluated the RNA-seq analysis of unaffected tissue compared with severely affected tissue. These data indicate severe wooden breast tissue has significant oxidative stress and hypoxia. However, this data does not elucidate possible causes of WB. The current study was conducted to detect changes in gene expression as the disease progresses. Therefore, at 42 d of age, 40 birds were euthanized and WB scores were determined by hand palpation (1 = none; 2 = mild, 3 = moderate; 4 = severe). Muscles samples were excised for RNA isolation followed by library preparation and Illumina HiSeq 2500 RNA sequencing at the Genomics Sciences Lab at NC State University. CLC Genomics Workbench (Qiagen) was used for mapping sequences to the genome and statistical analysis. A total of 142 genes were differentially expressed when comparing WB score 1 (none) to 2 (mild) with 86 being upregulated and 56 being downregulated. These data were then uploaded to Ingenuity Pathways Analysis (IPA) to analyze potential pathway regulation. The most effected pathways included calcium signaling, extrinsic prothrombin activation pathway, LXR/RXR activation, and FXR/RXR activation. These pathways are regulated by DNA methyltransferase (DNMT3A and DNMT3B) and TGFβ3. Moreover, there appears to be significantly more acute phase protein expression in breast tissue of mildly affected birds. As the disease progresses, the gene expression changes. When comparing WB 2 verses 3, new signaling pathways emerge including changes in Sirtuin signaling pathways that is involved in DNA repair and Leukocyte Extravasation Signaling pathways that are involved in movement of white blood cells out of blood into the extracellular space. Once birds have severe WB, the major altered pathways are RhoGDI signaling and actin regulation which likely reflect a physiologic endpoint. In conclusion, as the severity of WB changes so does the gene expression. At the onset of the disease, calcium signaling was most affected, but as the disease progressed, the genes that were changed indicated a potential cancer disease state.

Key Words: wooden breast, RNA seq, calcium signaling

233 Interaction of the macrophage migration inhibitory factor (MIF) with its putative receptors CXCR4 and CD74 and their complex. M. Park, S. Kim, and R. Dalloul*, *Virginia Tech, Blacksburg, VA.*

Macrophage migration inhibitory factor (MIF) is known as a chemokine-like inflammatory cytokine that regulates leukocyte migration and is involved in a wide variety of biological and pathological processes. The function of avian MIF in inhibiting the migration of chicken mononuclear cells while enhancing lymphocyte proliferation has been well documented. Efforts continue to characterize this pluripotent cytokine and its receptors in both host (chicken) and pathogen (*Eimeria*). Here, we further evaluated the interaction of avian MIF with its receptors CXCR4 and CD74. Receptor-specific transformants were constructed and the interactions tested using an array of assays with with chicken immune cells. Receptor binding tests included pull-down assay, co-immunoprecipitation, immunofluorescence, and flow cytometry. Data were analyzed by ANOVA and Tukey-Kramer multiple comparison test, and differences were considered significant at $P < 0.05$. Chicken MIF interacts not only with the cell surface receptor CD74 (ChCD74), but also with CXCR4 (ChCXCR4) as observed by immunofluorescence and flow cytometric analyses, as well as evidenced by an immunoprecipitated complex of ChMIF and ChCXCR4. ChMIF binding to ChCXCR4 promoted substantial internalization of ChCXCR4 in splenocytes, but

to a lesser extent in PBMCs and heterophils. Further, secreted MIF by the apicomplexan parasite *Eimeria* (EMIF) also binds to ChCXCR4 in a comparable fashion to ChMIF. ChCXCR4 binds to not only ChMIF and EMIF but it also interacts with ChCD74 as observed co-localization analysis. Overexpression of both ChCXCR4 and ChCD74 resulted in cell chemotaxis that abrogated inhibition of cell migration induced by ChMIF or EMIF. Conversely, cell migration was more impeded by ChMIF or EMIF as a result of blocking ChCXCR4 and ChCD74 than by treatment with MIF alone, corroborating the co-engagement of ChCXCR4 and ChCD74 in cell migration. A transient and rapid intracellular calcium influx was elicited upon stimulation with ChMIF or EMIF through ChCXCR4 alone or ChCXCR4 and ChCD74. ChMIF or EMIF-induced cell proliferation involving the interaction with ChCD74 was prominently enhanced when coupled with ChCXCR4. Collectively, these results support the functional responses followed by MIF interaction through CXCR4 and CD74 in regulating cell migration, calcium mobilization, and cell proliferation, implying that ChCXCR4 and ChCD74 are the effective receptors mediating the activity of avian and *Eimeria* MIFs.

Key Words: migration inhibitory factor (MIF), CXCR4, CD74, chicken, *Eimeria*

234 Divergent cellular fuel preferences in primary immune cells isolated from commercial type laying hens and broilers. E. Bobeck*, *Iowa State University, Ames, IA.*

It is well-documented that lymphocytes generally shift toward glycolysis during activation to promote inflammatory phenotypes and effector function. Advances in technology to understand immunometabolic shifts have made it possible to query the immune system on both a cell culture and whole-animal basis. The objective of this work was to compare baseline and stressed phenotypes of isolated primary peripheral blood mononuclear cells (PBMCs) isolated from laying hen and broiler strains. Using a Seahorse XFe24 Analyzer, baseline mitochondrial respiration of the cell (Oxygen Consumption Rate; OCR) and the baseline glycolytic activity (Extracellular Acidification Rate; ECAR) were compared directly, and then a metabolic pathway inhibitor was applied to determine metabolic capacity. Assay media concentrations and cell plating densities were optimized based on genetic strain in commercial lines (Ross 308; Bovans White; 25 mM glucose; 1 mM L-glutamine; 1 mM sodium pyruvate; 0.5uM FCCP; 1uM oligomycin). Results are representative of > 11 birds/strain and 3 replicate wells/bird, with means compared using Welch's *t*-test and significance reported at $P < 0.05$. Previous work in our lab has shown that at resting baseline, PBMCs from commercial laying hen and broiler lines displayed distinct preferences in pathway selection. Most notably, laying hen PBMCs at rest display a preference against using glycolysis, independent of cell plating concentration, versus broiler PBMCs (ECAR; 40.5, hen; 63.0, broiler; $P < 0.0001$). Mitochondrial respiration at baseline in hens showed a trend to be reduced compared with broilers (OCR; 226, hen; 301, broiler; $P = 0.0583$). While baseline fuel preferences were divergent, both laying hen and broiler-derived PBMCs maintained the same capability to respond via mitochondrial respiration to a pathway inhibitor drug challenge to force non-preferred pathway usage (OCAR; hen, 342; 413, broiler; $P > 0.05$). A vaccination challenge (BGG-conjugated peptide in complete Freund's adjuvant) did not change baseline pathway preference (ECAR = 292, non-vaccinated; 382, vaccinated; OCAR = 156, non-vaccinated; 64, vaccinated; $P > 0.05$) or metabolic potential in mitochondrial respiration, where non-vaccinated hens showed a 249% increase in mitochondrial pathway usage vs. 252% in vaccinated hens, and glycolytic capacity increased 213 and 207%, respectively in non-vaccinated vs vaccinated hens ($P > 0.05$). These results highlight distinct metabolic preferences for mitochondrial and glycolytic pathways in primary commercial layer and broiler PBMCs at rest based on genetic background. Forced pathway usage due to inhibitor drugs resulted in similar capacities to use mitochondrial-derived energy.

Key Words: PBMC, immunometabolism, laying hen, broiler, fuel preference

235 Effects of combination of antibiotic alternatives on multi-drug-resistant *Salmonella* Heidelberg in commercial turkeys. A. K. Johny*, D. V. T. Nair, J. V. Thomas, G. Dewi, T. Johnson, and S. Noll, *University of Minnesota, Saint Paul, MN.*

Salmonella enterica serovar Heidelberg (SH) can colonize the cecum of turkeys and disseminate to internal organs potentially compromising the microbiological safety of turkey products. With FDA's Veterinary Feed Directive in place, the search for new alternatives and refinement of the existing ones have taken priority. Our objective in the current study was to evaluate the effects of alternatives-to-antibiotics (A2A) interventions in combination against the emerging multidrug-resistant (MDR) SH in commercial turkeys. The A2A interventions included an autochthonous *Lactobacillus* probiotic (PRO), a mannooligosaccharide prebiotic (PRE), and a commercially available *Salmonella* vaccine (VAC). The treatment groups included in the study were: negative control (-SH, -PRO, -PRE, -VAC), SH control (+SH, -PRO, -PRE, -VAC), PRO group (+SH, +PRO, -PRE, -VAC), PRE group (+SH, -PRO, +PRE, -VAC), VAC group (+SH, -PRO, -PRE, +VAC), PRO+PRE group (+SH, +PRO, +PRE, -VAC), PRO+VAC group (+SH, +PRO, -PRE, +VAC), PRE+VAC group (+SH, -PRO, +PRE, +VAC) and PRO+PRE+VAC group (+SH, +PRO, +PRE, +VAC). Two independent experiments were conducted with 8 birds per group in each experiment (n = 72 turkeys/experiment). The turkeys were vaccinated (VAC) on d 1 as coarse spray and revaccinated at 3 weeks of age through drinking water. PRO (10^{10} cfu/ml) and PRE (0.2%) were supplemented to the turkeys through drinking water and feed for 7-weeks, respectively. Turkeys were challenged with a 2011 ground turkey outbreak strain of SH (10^6 cfu/turkey) at wk 6. Then, SH colonization and its dissemination to liver and spleen were determined after euthanizing the turkeys at 2 and 7 d post-inoculation (PI). The A2A interventions alone or in combination resulted in >1.0 $\log_{10}$ cfu/g reduction in cecal colonization of MDR SH and reduced its dissemination to liver and spleen ($P < 0.05$) after 2 and 7 d PI. The most consistent reduction obtained was with the vaccination treatments. Intervention with VAC resulted in 2.0 $\log_{10}$cfu/g reduction of SH in cecum at 2 d-PI ($P < 0.05$) whereas all VAC treatments completely inactivated cecal SH at 7 d-PI ($P < 0.05$). In addition, vaccination treatments completely inhibited MDR SH dissemination to liver and spleen at 2- and 7 d PI ($P < 0.05$). The results revealed that VAC and its combination with PRE and PRO could be effective strategies to control MDR SH colonization and its dissemination to internal organs in commercial turkeys, thereby potentially improving the microbiological safety of turkey products (UMN Rapid Response Fund Project# RR-212).

Key Words: *Salmonella* Heidelberg, probiotic, prebiotic, vaccination, antibiotic alternative

236 Exposure of a broiler to a xylanase for 35d increases the capacity of cecal microbiome to ferment soluble xylan. M. Bedford*[1] and J. Apajalahti[2], *[1]AB Vista, Marlborough, United Kingdom, [2]Alimetrics, Espoo, Finland.*

Inclusion of an xylanase in a broiler diet may select for a microbiome more capable of digesting xylose-rich carbohydrates. Broiler chickens were fed a wheat-soy diet which contained a xylanase (Econase XT, AB Vista, UK) at 0 or 16,000 BXU/kg for 35 days of age. At the end of this study the cecal contents from 10 individual birds from both groups were collected anaerobically as an inoculum to use in a fermentation study which started within 3 hours of collection of the last inoculum. Ileal digesta extract from the last third of the small intestine (5ml per in vitro incubation tube) from enzyme treated and untreated birds was added to 0.5g of cecal inoculum and 4.5ml of reduced anaerobic buffer to generate the control treatment. Additional treatments involved addition of 50 mg of either xylose and xylanase treated or untreated wheat NSP, to make 10 treatments in total to the enzyme untreated ileal digesta extract. Gas and SCFA production was monitored for 8 hours post-inoculation. Data were analysed by ANOVA and means separated by Dunnets test with p set at 0.05. Results showed that the substrate derived from the ileum of enzyme treated and untreated birds gave very similar results. This suggests that no additional soluble substrates were generated in the ileal digesta by the presence of the enzyme which would drive greater levels of SCFA or gas in the ceca. Gas production after 3 hours was not enhanced by addition of any of the xylose rich amendments to the control bird cecal inocula, in contrast to the large (up to 28%) increment in gas production with the cecal inocula from birds pre-exposed to the enzyme. SCFA changes indicated that pre-exposure of the inocula to the xylanase resulted in less acetate and more butyrate being produced on addition of the xylose-containing additives compared with the unexposed control. These data suggest that the exposure of a broiler to a xylanase augments the capacity of the microbiome to utilise soluble xylose-rich carbohydrates which has implications for intestinal health and energy extraction from the diet in older animals.

Key Words: xylanase, ceca, fermentation

237 The impact of metabolizable energy, amino acid density, and starch to lipid ratio on growth and carcass parameters of male broilers from 21 to 35 days of age. S. Liu*[1], V. Naranjo[2], A. Moss[1], and P. Selle[1], [1]*The University of Sydney, The Oaks, NSW, Australia,* [2]*Evonik Nutrition & Care, GmbH, Hanau-Wolfgang, Germany.*

Understanding the responses to metabolizable energy (ME) and amino acid (AA) density levels are critical to optimize growth performance of modern broilers. However, additional factors may interact with ME and AA levels in driving feed intake and hence growth performance. Therefore, a 3-factor, 3-level Box Behnken design was used to determine the impact of nitrogen corrected ME (AMEn, 11.25, 12.38, 13.50 MJ/kg), standardized ileal digestible Lys (SID Lys, 9.20, 10.65, 12.10 g/kg) and starch to lipid ratio (S:L, 4.50, 12.25, 20.00 g/g) on growth and carcass parameters of male broilers from d 21 to 35. A total of 1,950 d-old Ross 308 male broilers were assigned to 13 dietary treatments (pellet form) with 10 replicates of 15 birds per replicate pen. Broilers were fed the same nutritionally adequate diets until d 20. Experimental diets were based on corn, soybean meal and wheat middlings with varying additions of corn gluten meal, soy protein concentrate and corn starch. Diets were formulated to meet or exceed the ideal AA profile according to AMINOChick 2.0. On d 35, 4 birds per pen were selected based on average pen weight for carcass measurements. Response surface was fitted by first- and second-degree polynomial regressions in R 3.1.3. In the predicted model, the non-significant coefficients were excluded for recalculations of the reduced equations for each response variable. Feed intake (FI) was affected by SID Lys (linear and quadratic, $P < 0.01$), AMEn × SID Lys ($P < 0.01$) and AMEn × S:L ($P < 0.01$). Pellet durability index varied across treatments and was linearly associated with feed intake ($R^2 = 0.78$, $P < 0.001$). Increased AMEn levels decreased feed intake but this response was greater at higher SID Lys levels and at higher S:L ratio. Body weight gain (BWG) was affected by SID Lys (linear, $P < 0.01$) and S:L ratio (linear and quadratic, $P < 0.01$). BWG improved with increasing levels of SID Lys and was reduced with increasing S:L ratio. No effect ($P > 0.05$) of AMEn on BWG. Feed conversion ratio (FCR) was affected by SID Lys (linear and quadratic, $P < 0.01$), AMEn (linear and quadratic, $P < 0.01$) and S:L (linear, $P < 0.01$). Increasing levels of SID Lys and AMEn improved FCR, while increasing S:L slightly impaired FCR. Carcass weight linearly improved with increasing SID Lys ($P < 0.01$) and linearly reduced with increasing S:L ratio. Results from this experiment indicate that modern male broilers are highly responsive to increasing AA density levels (SID Lys), even above current recommendations, improving BWG, FCR and carcass weight independent of AMEn and S:L levels while these factors interact in driving FI.

Key Words: broiler chicken, lysine, metabolizable energy, starch, lipid

238 Evaluation of amino acid density on growth performance and carcass yield in 33 day old broilers. R. Shirley*[1], B. Bodle[2], R. Latham[3], R. Brister[3], and J. Lee[2], [1]*Adisseo USA, Alpharetta, GA,* [2]*Texas A&M University, College Station, TX,* [3]*Tyson Foods Inc., Springdale, AR.*

In a 3-phase, 33 d-old, small-bird program, the growth performance and carcass yield of Cobb 500 × M99 mixed-sex broilers were evaluated after altering the dietary AA densities in the last 2 feeding phases. The experimental design consisted of 10 dietary treatments (trts) that were allotted in a randomized, complete block design, with 13 replicate pens/ trt and 25 birds/ replicate pen. A common starter was fed for 12 d; however, in the grower (fed 13–26 d-of-age) and finisher (fed 27–33 d-of-age) phases, a combination of 4 dAA densities was fed. All trts within a given phase were formulated to be iso-caloric, and dEAA: dLys ratios were maintained across all dietary treatments within a given phase. In the grower and finisher phases, the respective dLys values in the control diet were 1.05% and 0.92%; the control diets represent trt 1. In the grower and finisher phases, the following adjustments were made against the dLys in the respective control feeds: −5% (trt 2), +5% (trt 3), and +10% (trt 4). These diets were fed throughout the grower and finisher phases. In the grower phase, diet 1 was fed to trts 5, 6 and 7; and, diets 2, 3 and 4 were fed to trts 8, 9 and 10. In the finisher phase, diets 2, 3 and 4 were fed to trts 5, 6 and 7; and, diet 1 was fed to trts 8, 9 and 10. On d 34, following an 8 h feed withdrawal, 7 broilers per replicate pen were selected and processed to determine weight and yield of the carcass and fat pad. All data were subjected to a one-way ANOVA, with means deemed significant at $P \leq 0.05$; significant means were separated using Duncan's Multiple Range Test. Body weight between the experimental treatments was not significantly impacted ($P = 0.08$) at d 33; however, the reduction in AA density resulted in an overall increase in feed consumption ($P < 0.001$), which negatively impacted mortality-adjusted feed conversion ratio (FCR; $P < 0.001$). The incremental increase in dAA density among the first 4 treatment groups resulted in significant, stepwise increases in dLys intake and stepwise reductions in FCR. This effect was also observed in the grower and finisher phases for trts 5 – 7 and 8 – 10. Compared with the control, there was a trend for increasing carcass yield in birds fed the −5% dLys trts; however, supplementing dLys at +5 and +10% increased carcass yield ($P < 0.05$) and reduced fat pad yield ($P < 0.05$). Linear regression with all 10 treatment groups indicates a positive impact on FCR and carcass yield with increasing dLys intake. These data demonstrate the importance of dLys intake on growth performance and carcass yield.

Key Words: amino acid density, broiler, carcass yield

239 Protein reduction in broiler diets allowed for high performance with improved footpad quality while litter volume and N-excretions were decreased by more than 25%. P. Hiller[2], I. Simon[3], V. Taube[4], M. Klahsen[2], J. Stegemann[3], and A. Lemme*[1], [1]*Evonik Nutrition & Care GmbH, Hanau, Germany,* [2]*Chamber of Agriculture Lower Saxony, Oldenburg, Germany,* [3]*Chamber of Agriculture North Rhine-Westphalia, Bad Sassendorf, Germany,* [4]*BEST 3 Geflügelernährung GmbH, Twistringen, Germany.*

In the light of recently revised German legislation on on-farm nitrogen (N) management, the impact of decreasing dietary N-load at balanced dietary amino acid supply on growth performance and N-excretions was examined in 5000 as-hatched Ross 308 broilers 1 to 40 d of age (20 floor pens with 250 birds each, bedding: 70kg straw pellets; no further bedding in the course of trial). In addition to diets representing the German standard with 22.0, 20.6, 20.0, and 19.5% crude protein (CP) in starter (1–10d), grower I (11–16d), grower II (17–30d), and finisher feed (31–40d), a second set of N-reduced diets recommended by German DFG (21.0, 20.0, 19.6, and 18.9% CP) was fed in treatment 2. Dietary CP was further reduced in treatment 3 (21.0, 19.5, 18.7, and 18.0% CP) and treatment 4 (21.0, 19.0, 18.0, and 17.0% CP). Growth performance, feed conversion (FCR), carcass quality, N-utilization, litter quantity (at termination) and economic performance were examined and analyzed by one-way ANOVA with subsequent Tukey test. Final body

weight (2808[a], 2782[ab], 2792[a], 2734g[b]) were similar between treatments 1, 2, and 3 but slightly impaired in treatment 4 while FCR (1.61, 1.63, 1.61, 1.64kg/kg) was not affected. This was reflected in the production efficiency factor (424[a], 413[ab], 426[a], 405[b]). Mortality amounted to 2.48, 2.96, 1.68, and 2.64%. Dietary N-reduction resulted in gradually improved N-utilization (58[d], 60[c], 63[b], 65%[a]) resulting in reduction of N-excretions in treatments 2, 3, and 4 by 9, 19, and 26% (54.7[c], 48.6[b], 44.4g/bird[a]) compared with treatment 1 (60.1[d]). Moreover, quantity of litter at termination of the trial was 8, 21 and 27% lower in treatments 2, 3, and 4 compared with treatment 1. Also, dry matter content of litter increased with dietary N-reduction. Carcass evaluation based on 25 birds per sex and treatment provided indifferent data for male and female birds. Carcass yield and breast meat yield did not differ significantly between treatments in male broilers while carcass weight and breast meat weight differed particularly between treatment 1 and 2 ($P < 0.05$). In female broilers, carcass yield was similar between treatments 1, 2, and 3 (71.0[a], 71.3[ab], 70.4[ab]) whereas it was lower in treatment 4 (70.0%[b]). Breast meat yield (% of carcass) did not differ between treatments ($P > 0.05$). Percentage of chicken footpads with no lesions (score 0) almost doubled (treatment 3) or tripled (treatment 4) compared with treatment 1. Economic calculations using diet prices and other relevant cost and income values suggested that treatment 3 was competitive to the standard feeds but offered advantages concerning environmental and chicken welfare aspects.

Key Words: broiler, low protein diet, performance, nitrogen balance, economics

240 Threonine requirements of broilers based on a stochastic approach. G. Viana*[1], N. Sakomura[1], and M. Reis[2], *[1]UNESP, Jaboticabal, Brazil, [2]UNESP, Jaboticabal, Brazil.*

Factorial models are a long-established approach, which accounts maintenance and growth needs to determine broiler requirements for essential amino acids. Despite being effective in predicting broiler requirements irrespective of the genetic, sex, body composition, and environment, factorial method is often criticized for being deterministic, i.e., its estimates represent only the average individual in a population and thus, does not consider the inherent variability in genetic growth potential. By considering such genetic variation in a population, it is possible to transform deterministic factorial models on stochastic, whose predictions are more realistic. The procedures adopted herein aimed to introduce the concept of stochasticity in a factorial model developed to predict broiler requirements for threonine (Thr). First, a variation (standard deviation) were introduced in Gompertz parameters determined in a study conducted at FCAV/UNESP. Such parameters and their respective standard deviations were: W*i* (initial body weigh = 43.7 ± 1.9), W*m* (mature body weight = 8.376 ± 0.135) and B (maturity rate = 0.04 ± 0.001). Once established the broiler growth model and based on the growth variation, a population of 500 individuals (broilers) were generated using the random function of Excel. Body weight of each individual was predicted and such data were introduced as inputs of a factorial model for Thr generated at UNESP/FCAV Brazil. From daily BW data it was calculated daily body weight gain (BWG). Both inputs were used in the following factorial model: Thr intake (mg/bird/day) = $(21.9 \times BW^{0.75}) + [(- 11.15 + 9.13 BWG)/k]$, where *k* is the efficiency of Thr utilization (0.73). The model was applied in the feeding program of 4 phases: 1–7d, 8–21d, 22–33d, and 34–42d of age. Threonine requirements (mg/bird/day) with their respective standard deviation for these phases were estimated in 206 ± 11.2 (1 – 7d), 651 ± 38.5 (8 – 21d), 1269 ± 63.7 (22 – 33d), and 1542 ± 56.2 (34 – 42d). Stochastic approach has proved to be a useful tool to understand the variation in broiler growth potential and broiler Thr requirement.

Key Words: amino acid, factorial method, Gompertz function, growth model, stochasticity

241 The effect of dietary protein and amino acids on gene transcription during the progressive development of the "woody breast" myopathy in commercial broiler chicks. J. Griffin, M. Wick, L. Moraes, and M. S. Lilburn*, *Ohio State University, Wooster, Ohio.*

The "white striping/woody breast" muscle myopathy is a progressive myopathy that often begins with the visual appearance of white stripes followed by varying degrees of superficial and progressively deeper muscle hemorrhages and collagenous infiltration near the thickened, caudal region of the breast muscle. Much of the research on the etiology of this myopathy has utilized normal and myopathic muscle samples from older birds and there has been little data with younger chicks during the early stages of muscle growth. Griffin et al. (2017) developed a unique ranking system for documenting the ontogeny of WS/WB in broiler chicks from 3 to 46 d of age. A statistical model was subsequently developed that described the physical aspects of breast muscle growth (i.e., length, width, weight, depth) which had the greatest predictive value relative to the onset of WS/WB. The chicks used in this experiment were fed one of 3 diets formulated to commercial crude protein and amino acid levels (PC) and 2 negative control treatments containing approximately 4–5% (NC1) and 8–10% (NC2) reductions in amino acid levels. At each age, RNA was extracted from individual breast muscle samples and subjected to NanoString proprietary analysis which quantifies transcript abundance for selected genes normalized to a minimum of 3 reference genes. The data was analyzed using 2 complementary approaches: i) local regression (loess) was used to investigate the form of the relationship between transcript abundance and age, conditional on treatment and ii) a linear mixed effects model was used to investigate the systematic effect of age and treatment, as well as a potential interaction, on the gene transcripts using a continuous autoregressive correlation structure for the errors. Loess analysis suggested that transcripts of most genes were, as expected, affected by bird age and the form of the relationship varies considerably across genes. A pool of genes was affected by the diet but the interaction effect was often significant ($P < 0.05$), suggesting that the treatment effect was dependent on chick age. Dietary protein/amino acid effects on 17 transcripts were identified at varying ages for different genes (e.g., MTORC1, TNFα, Cemb1). The diet by gene transcription effects were observed beginning very early in the experiment through 46 d. In summary, broiler breast muscle gene transcription, like other aspects of growth, exhibits a temporal pattern that will respond to dietary changes in amino acids. These differences in gene transcription are likely the response to diet and diet related effects on muscle growth and the subsequent onset of the WS/WB myopathy.

Key Words: woody breast, breast muscle, amino acids, transcription, age

242 Bioavailability of DL-methionine hydroxy analogue relative to DL-methionine and validation of the multi exponential regression approach by using 65%-diluted DL-methionine in broilers. V. Naranjo*[1], A. Lemme[1], P. Agostini[2], and P. van der Aar[2], *[1]Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany, [2]Schothorst Feed Research, Lelystad, the Netherlands.*

Knowledge on the nutritional value of nutrient sources (relative bioavailability, RBA) and their respective replacement ratio is critical for cost effective formulation of broiler diets. Dose-response trials with subsequent multi-exponential regression analysis have been widely used for various nutrient sources and in different species. This applies also for the methionine (Met) sources DL-Met hydroxy analog free-acid (liquid MHA-FA) and DL-Met (DLM). However, suitability of the mathematical approach has always been a matter of discussion. Therefore, a broiler feeding trial was conducted to determine the RBA of MHA-FA relative to DLM for common performance parameters. To examine the suitability of the multi-exponential regression analysis for estimating the RBA 65%-diluted DLM (DLM65) was used as an internal standard. RBA of DLM65 is a priori assumed to be close to 65% because of its respective dilution. A total of 1,920 d-old male Ross 308 were fed starter (d 0 to 11), grower (d 11 to 28), and finisher diets (d 28 to 35). Each phase comprised 16 treatments (6 replicates of 20 birds per pen) including a basal diet deficient in digestible (dig.) Met+Cys without supplemental Met; and 5 increasing levels of either DLM, MHA-FA and DLM65. Met sources were added in all phases on weight/weight basis at 0.04, 0.08, 0.12, 0.21 and 0.30%. The dig. Met+Cys levels of the starter, grower, and finisher basal diets were 0.617, 0.535 and 0.505%, respectively. Growth performance parameters were evaluated for each phase and carcass evaluation was determined on d 35. Data were subjected to multi-exponential regression analysis in Genstat using the nonlinear-regression procedure. Analyzed total Met values from all dietary treatments were in line with calculated values. Overall (d 0 to 35) increasing levels of all Met sources improved ($P < 0.05$) body weight gain (BWG), FCR, European production efficiency factor (EPEF), carcass yield (CY) breast meat yield as % of BW (BMY-B), and as % of CY (BMY-C) compared with the basal diet. The estimated RBA of MHA-FA were 58, 66 and 62% for BWG, FCR and EPEF while for DLM65 were 56, 54, and 59%, respectively compared with DLM. For CY, BMY-B, BMY-C the estimated RBA of MHA-FA were 63, 65, and 73% while for DLM65 were 58, 65 and 73%, respectively, compared with DLM. Confidence intervals confirmed that all RBA estimates of MHA-FA were significantly lower than 88%. In conclusion, the average RBA of MHA-FA and DLM65 were 64 and 61% respectively compared with DLM. The results demonstrate that the RBA of MHA-FA is significantly lower than its active content of 88% and that the multi-exponential regression analysis is a suitable methodology to estimate the bioavailability of nutrient sources.

Key Words: bioavailability, bioefficacy, broiler, methionine, methionine hydroxy analogue

243 Digestibility of guanidinoacetic acid is 100% in broilers while availability depends on dosage and dietary arginine supply.
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Creatine is an essential compound in cellular energy metabolism of vertebrates. Poultry diets often contain little to no creatine-containing ingredients, so broilers depend largely on *de-novo* formation of guanidinoacetic acid (GAA) from arginine (Arg) and glycine and its subsequent methylation to creatine. Previous research showed that dietary Arg level influences the efficacy of dietary GAA supplementation. In the present study, a total of 280 broilers (56 cages × 5 birds) were assigned to 7 dietary treatments comprising an Arg-deficient, all-vegetable basal diet (diet 1), 2 diets supplemented with 0.16% (diet 2) or 0.32% (diet 3) L-Arg, and 2 inclusion levels of GAA (0.06%, 0.12%) to diets 1 (diets 4, 5) and 2 (diets 6, 7), respectively. Birds were fed experimental diets from 8 to 22 d post-hatch. Ileal digesta and excreta (48-h collection) samples were collected at study conclusion. The indicator method (TiO_2 as indigestible marker) was applied for calculating GAA digestibility for GAA-supplemented diets (diets 4–7) and diets 1–3 were used to quantify basal endogenous losses of creatine-related metabolites (GAA, creatine, creatinine). Although interactive effects existed ($P < 0.05$), apparent and true ileal GAA digestibility was complete in all treatments (diets 4–7) and differences between treatments were numerically small. Increasing levels of both GAA and Arg reduced ($P < 0.05$) apparent and true GAA excreta digestibility, possibly due to the contribution of GAA in urine, which is increasing with increasing Arg in the base diets (diet 1–3). Comparing diets 1–3 showed that increasing dietary Arg particularly increased ($P < 0.01$) endogenous GAA losses in excreta, as these levels were 24- to 164-fold higher than in ileal samples, thereby suggesting increased de novo GAA formation and concomitant urinary losses. Because of such additional excretions, excreta digestibility is considered inappropriate for assessing GAA digestibility in birds. All creatine-related metabolites (GAA, creatine, creatinine) were considered for assessing true ileal and excreta GAA availability (i.e., GAA retained in the body) in diets 4–7 and corrected for base levels from respective samples in unsupplemented diets 1 and 2. Calculated true ileal GAA availability ranged between 117% and 131%. True excreta GAA availability was 107, 86, 90, and 70% for diets 4–7, respectively, as influenced by dietary Arg ($P < 0.01$) and GAA ($P < 0.01$) supply. Therefore, assessing availability using excreta samples was considered the appropriate approach because both fecal and urinary sources were included. We conclude that GAA is fully digestible while availability depends on dietary Arg status as well as on dosage of dietary GAA.

Key Words: broiler, guanidinoacetic acid, arginine, digestibility, availability

244 Amino acid requirements of high yield broiler breeders to 40 weeks of age, reared on low and high weight profiles.
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There is a lack of research on high yield broiler breeders. Further, breeder research with Cobb 700s is sparse. The present study was designed to evaluate the protein requirements of the Cobb 700 broiler breeder and specifically to clarify the BW and amino-acid (AA) requirements of the Cobb 700 broiler breeders. Our hypotheses were that heavier pullet/hen BW and/or elevated dietary AA for the broiler breeder hens would increase the number and weight of hatching eggs per hen housed (HEHH). A total of 4,640 Cobb 700 females that were placed randomly at day one in 16 pens (8 per treatment; proposed (P) BW and control (C) BW) and fed common diets to achieve BW per assigned treatment. At 22wk of age, 2,040 pullets per rearing treatment were randomly divided into 24 pens and then fed respective low (L) or high (H) AA feeds (LAA = 14.00% CP of diet, Lys = 0.59%, Met = 0.30%; HAA = 15.02% CP of diet, Lys = 0.72%, Met 0.37%). The experimental design consisted of 2 pullet treatments × 2 hen feed treatments, for a combination of 4 pullet/hen treatments. Random Block Design ANOVA was performed using JMP Pro 13 software, and means were separated by Student's t-Test. Rearing BW target differences ($P \leq 0.05$) were achieved by 4 wk of age and maintained with the P treatment having heavier weights ($P \leq 0.05$) until 27 wk of age. However, by 28 wk of age pullet rearing treatment differences ($P > 0.05$) diminished. At 28 wk there was an effect on BW from the AA treatments where the HAA treatments had

higher BW ($P \leq 0.05$). Early egg production to 35 wk was increased ($P \leq 0.05$) from hens from the heavier pullet treatment groups, but were unaffected by hen diets. After 30 wk there was no effect ($P > 0.05$) on egg production by pullet treatment or hen diet treatments. In contrast, egg weights were increased ($P \leq 0.5$) in hens receiving the higher dietary levels of AA beginning at wk 38, but were unaffected by pullet treatment. At 38 wk of age, feathering scores were decreased ($P \leq 0.05$) in hens receiving the higher AA diets. In conclusion, there may be some benefit in having heavier pullet rearing weights on early egg production with higher hen AA diets influencing egg and feather quality, but further research is warranted.

Key Words: broiler breeder, amino acid, Cobb 700

245 Low crude protein diets for laying hens: Effect of dietary tryptophan and valine level on production performance. L. Star*[1], J. Ensink[2], M. Rovers[2], W. Lambert[3], and E. Corrent[3], *[1]Schothorst Feed Research, Lelystad, the Netherlands, [2]Orffa Additives B.V., Werkendam, the Netherlands, [3]Ajinomoto Eurolysine S.A.S., Paris, France.*

For laying hens the reduction of crude protein (CP) is of interest if amino acids (AA) will not become limiting. Therefore, a trial was performed to test the effect of a low CP diet with added L-tryptophan (Trp) and L-valine (Val) on production performance of Bovans Brown laying hens from 28 to 39 weeks of age. The trial was conducted with 5 treatments and 12 replicates with 12 birds each. Treatment 1 (positive control, PC) received a diet with 16.2% CP and a digestible Trp-to-digestible lysine ratio (dTrp:dLys) of 25% and digestible Val-to-digestible lysine ratio (dVal:dLys) of 100%. Treatment 2 (negative control, NC) received a diet with 13.5% CP and a dTrp:dLys of 19% and dVal:dLys ratio of 79%. Treatments 3 and 4 received diets with added L-Trp and L-Val, respectively, to a dTrp:dLys and dVal:dLys ratio comparable to the PC diet. For Treatment 5 the diet was added with both L-Trp and L-Val to dTrp:dLys and dVal:dLys ratios comparable to the PC diet. Measured parameters were feed intake, laying rate, egg weight, egg mass and feed conversion ratio (FCR). During the first weeks, feed intake was that low that birds were not able to increase laying rate and egg weight. Birds had to recover thereafter. Trial weeks 9-12 were representative for the effect of dietary Trp and Val levels on performance. Addition of L-Val to the NC diet resulted in a higher egg weight compared to the NC treatment ($P < 0.05$) and numerically higher than the PC treatment. Addition of L-Trp to the NC diet resulted in the highest laying rate and highest egg mass, resulting the lowest FCR after the PC treatment ($P < 0.05$). Addition of both AA to the NC diet was less effective and performance was only slightly improved compared to the NC treatment. In conclusion, this trial indicates that CP in layer diets can be significantly reduced without affecting production performance if daily intake of AA is covered.

Key Words: tryptophan, valine, requirement, ratio, laying hen

246 Effects of arginine supplementation on growth performance and physiological traits in broiler chickens raised under different stocking densities. D. H. Kim[2], M. C. Keum[2], S. Lee[2], H. Lillehoj[1], and K. W. Lee*[2], *[1]USDA, Beltsville, MD, [2]Konkuk University, Seoul, Korea.*

This study was conducted to investigate the effects of stocking density and arginine on growth performance, tibia bone quality, serum parameters, physiological stress indicators, intestinal volatile fatty acids, and gut health in broiler chickens. A total of 840 one-day-old feather-sexed male broiler chicks were randomly allocated to 6 treatments in a 3 × 2 factorial arrangements, including 2 stocking densities (7.5 birds/m^2 [LSD], 12.5 birds/m^2 [HSD]) and 3 levels of arginine (100 [ArgS], 150 [ArgM], and 200% [ArgH] of NRC recommendations). Experiment lasted for 35 d. All data were analyzed by 2-way ANOVA with stocking density and dietary arginine as main factors. Duncan's multiple range test at a 5% probability was used for comparison between means. Average daily weight gain was improved ($P < 0.01$) in LSD- vs. HSD-housed chickens from 21 to 35 d and from 1 to 35 d. An average daily feed intake was increased ($P < 0.01$) in LSD-housed chickens compared with HSD-housed chickens at all ages. Depression in feed conversion ratio and average daily feed intake were noticed in ArgM- and ArgH-fed groups compared with ArgS-fed groups from 1 to 21 d of age. Tibia length was increased ($P < 0.05$) in LSD-housed chickens compared with HSD-housed chickens at d 35. No significant difference was noted in the concentration of ileal secretory immunoglobulin A. The concentration of glutamic oxalacetic transaminase was lowest ($P < 0.01$) in ArgS-fed groups. Biological stress indicators were not affected by any of the treatments. Significant interaction ($P < 0.05$) between arginine level and stocking density on jejunal volatile fatty acid levels was noted. Collectively, the findings of this study suggest that dietary arginine did not alleviate the stocking density-induced growth depression in broiler chickens.

Key Words: stocking density, arginine, growth performance, stress indicator, broiler

247 Meta-analysis of broiler studies shows that *Bacillus subtilis* DSM 32315 can improve bird performance similar to an antibiotic growth promoter. A. Sokale*, *Evonik Corporation, Kennesaw, GA.*

Intensive poultry production often predispose birds to conditions which can alter the gut microbiota and increase their susceptibility to enteric disease, resulting in loss of performance. Sub-clinical enteric diseases, which are often unnoticed, can negatively impact performance and cause economic losses to producers. Direct-Fed microbials or Probiotics are increasingly more successful in commercial poultry production due to their ability to support intestinal microbiota balance, improving gut health and performance of birds. In 8 independent trials, the effect of *Bacillus subtilis* DSM 32315 (*B. subtilis* DSM 32315) on the performance of broiler chickens raised under challenging environmental (used litter and heat stress) and dietary (meat and bone meal, and distillers dried grains with solubles) conditions, was tested. It was hypothesized that inclusion of *B. subtilis* DSM 32315 to broiler diets could improve broiler performance under various production conditions. All trials were conducted in floor pens from either d 0 to 35 or d 0 to 42. In each trial, *B. subtilis* DSM 32315 and antibiotic growth promoter (AGP) were independently compared with the non-medicated control group (NMC) for the evaluation of body weight (BW), adjusted feed conversion ratio (aFCR), and mortality. The standard difference in means and 95% confidence interval were used for the analysis. The average percent change in BW, aFCR, and mortality between NMC vs. AGP, and NMC vs. *B. subtilis* DSM 32315 was calculated and reported. In comparison to NMC, AGP and *B. subtilis* DSM 32315 increased BWG of birds by 31g and 36g, respectively. In addition, AGP and *B. subtilis* DSM 32315 decreased aFCR of birds by 3.1 and 3.3 percentage points, respectively, over the NMC group. Similarly, AGP and B. subtilis DSM 32315 lowered mortality by 1.71% and 2.08%, respectively, in comparison to NMC. In conclusion, in various simulated commercial grow-out conditions presenting mild enteric challenges to broiler chickens, the supplementation of *B. subtilis* DSM 32315 significantly ($P \leq 0.05$) improved growth performance in a manner that is similar to AGP.

Key Words: broiler, direct-fed microbial, meta-analysis, mortality, performance

248 Standardized phytomolecules successfully replace avilamycin in broiler raised under commercial-like conditions. J. Grandmaison* and J. S. Casahualpa, *Pancosma SA, Geneva, Switzerland.*

Routine inclusion of antibiotic growth promoters (AGP) in the diet of commercial livestock continues to be standard practices in many countries. Among them Avilamycin is one of the most well-known. Plant extracts have shown inconsistent results when compared with AGP in research stations, limiting their interests for livestock industrials. The objective of this trial was to evaluate if blends of standardized phytomolecules could efficiently replace Avilamycin in broilers housed in commercial situations. Day-old-broilers Cobb 500 (n = 1080) were allotted to 3 groups of 360 birds and assigned into 6 replicates for 42 d. Pens were allocated into a broiler commercial farm. Following feed treatments were applied: CONTROL - No supplementation; AVILAMYCIN - Avilamycin at 100 g/t; XT – from d 1–21 XTRACT Nature (4% capsicum + 4% turmeric oleoresins, Pancosma, Switzerland) at 100 g/t and from d 22–42 XTRACT 6930 (5% carvacrol, 3% cinnamaldehyde, 2% capsicum oleoresin, Pancosma, Switzerland) at 100 g/t. Growth performance and histological parameters were measured. Data were analyzed using mixed model of XLSTAT. Broilers from XT group performed better than broilers from CONTROL and AVILAMYCIN groups. From 0 to 42 d, they had significantly lower feed conversion ratio in comparison to CONTROL ($P = 0.047$) and numerically lower than AVILAMYCIN. Final Body Weight (BW) of XT broilers was heavier (2.589 kg) than final BW of CONTROL broilers (2.527 kg; $P = 0.071$) or AVILAMYCIN broilers (2.568 kg). Enterocytes integrity of XT broilers were significantly improved in the jejunum ($P = 0.047$) and the ileum ($P = 0.012$) in comparison to the 2 other groups. Jejunum villus height of broiler feed XT were also significantly greater (184.2 µm) than jejunum villus of broiler feed CONTROL and AVILAMYCIN (173.3 and 176.7 respectively, $P < 0.001$). These histological indicators are signs of better intestinal health. These findings confirm that standardized phytomolecules, used at the right dose and at optimal physiological stage could efficiently replace avilamycin in broiler housed in commercial like conditions.

Key Words: phytomolecule, broiler, performance, intestinal health, antibiotic

249 Effect of dietary protein source and *Bacillus subtilis* probiotic (Alterion) supplementation on growth performance and meat yield of broiler chickens reared to 46 days of age. C. Starkey*[1], R. Shirley[2], A. Welsher[2], O. Tejeda[1], L. Spencer[1], and J. Starkey[1], *[1]Auburn University, Auburn, AL, [2]Adisseo USA Inc., Alpharetta, GA.*

The objective of this 3 × 2 factorial study was to determine whether the growth and meat yield of broilers that were hatched and reared without antibiotics differed when fed: 1) 3 different dietary protein sources and 2) with or without supplemental *Bacillus subtilis* probiotic (PROB; Alterion; DSM 29784; Adisseo USA). To investigate this, 3 dietary treatments were formulated to include either: 1) soybean meal (SBM), 2) poultry by-product meal + feather meal (50:50 blend; PBM) or 3) mammalian meat and bone meal (MBM). All diets were formulated to meet or exceed the nutrient recommendations of the primary breeder and were both iso-caloric and iso-nitrogenous. Diets that contained either SBM, PBM or MBM were either: 1) not supplemented with the *Bacillus subtilis* (-PROB) or 2) supplemented with the *Bacillus subtilis* (+PROB). Upon arrival, day-of-hatch, male Yield Plus × Ross 708 broiler chicks (n = 1,800) were randomly assigned to 1 of the 6 treatments; each treatment was replicated across 12 pens, with 25 birds per 2.3 mm^2 floor pen. In each litter treatment, broilers received 1 of 6 diets in 4 phases: starter (d 1 to 12), grower (d 13 to 27), finisher (d 28 to 38), and withdrawal (d 39 to 46). Mortality-adjusted BW, feed intake (FI), and FCR were determined for each phase. On d 47, following a 24-h static water chill, broiler carcasses were deboned and part yields were determined. Data were analyzed using the MIXED procedure of SAS. Means were considered significantly different when $P \leq 0.05$. While d 0 to 46 broilers fed PBM tended to have lower BW gain compared with those fed MBM or SBM ($P = 0.053$), FCR was similar among protein sources ($P = 0.437$). Broilers fed MBM and SBM yielded heavier carcasses ($P = 0.0149$) with less abdominal fat ($P = 0.0015$), and had heavier breast fillets ($P = 0.005$) and tenders ($P < 0.0001$) compared with those fed PBM. Supplementing the PROB significantly improved broiler BW gain and FCR from d 0 to 12 ($P < 0.0001$) and from 0 to 27 ($P \leq 0.0292$). Supplementing PROB in the starter phase significantly improved the BW gain of broilers fed MBM and SBM ($P = 0.0018$);

however, it did not alter BW gain in broilers fed PBM. Furthermore, broilers fed SBM and PROB during the starter and grower phases had lower FCR than those fed SBM alone ($P = 0.0357$). In the finisher phase (d 0 to 38), broilers fed PROB and either MBM or SBM had lower FCR than those receiving no PROB ($P = 0.0171$). No difference in FCR was detected between broilers fed PMB, with or without PROB. Overall, the results of this study indicate that supplementation of broiler diets containing different protein sources with this *Bacillus subtilis* probiotic can positively impact growth performance and feed efficiency.

Key Words: broiler chicken, protein source, *Bacillus subtilis* probiotic, growth performance, carcass yield

250 Use of Alquernat Nebsui (botanical product) as an alternative to an antibiotic growth promoter in broilers. J. P. Orpí*[1], A. Tesouro[1], and M. Rosemberg[2], *[1]Biovet, S.A., Constantí, Tarragona, Spain, [2]Universidad Científica del Sur (UCSUR), Lima, Peru.*

Alquernat Nebsui is a natural product based on active botanical molecules called pronutrients that improve intestinal physiology to optimize feed utilization and, consequently, increase productive performance. An experiment was conducted to evaluate whether Alquernat Nebsui could replace an antibiotic growth promoter (AGP) in broilers through the analysis of growth performance and the intestinal relative weight (as an indicator of intestinal efficiency). The trial was carried out in an experimental poultry unit with 300 one-day-old broilers of the Cobb 500 strain. Chickens were raised for 42 d and allotted to 4 treatments: 1) CN (basal diet without growth promoters); 2) BA (basal diet + bacitracin methylene disalicylate at 0.5 kg/ton, continuously, complying with the withdrawal period); and 3) AN (basal diet + Alquernat Nebsui at 0.5 kg/ton, continuously during all the trial). There were 10 replicate pens per treatment and 10 broilers per pen. Feed intake, body weight and feed conversion rate (FCR) were evaluated weekly. Efficiency index and relative intestinal weight were evaluated at the end of the trial. AN obtained the best FCR (1.56), followed by BA (1.58) and CN (1.61), with significant differences between AN and CN ($P < 0.05$). No significant differences were observed in the other parameters (feed intake, final body weight and efficiency index), although AN and BA obtained better results than CN (regarding final body weight and efficiency index). Relative intestinal weight was lower in AN, compared with the other groups, which is correlated with a better efficiency of the organ. Dietary supplementation of Alquernat Nebsui can better feed conversion rate and improve the physiology of the digestive tract in broilers. Results suggest that this product can be used as a natural growth promoter to replace AGPs.

Key Words: pronutrients, growth promoter, broiler, growth performance, gut efficiency

251 Effect of an organic acid drinking water acidifier on the growth performance, feed efficiency and footpad quality of broiler chickens raised under typical US production conditions. G. Page*[1] and Y. Han[2], *[1]Trouw Nutrition Agresearch, Guelph, ON, Canada, [2]Trouw Nutrition, Amersfoort, the Netherlands.*

As the poultry industry continues to reduce antibiotic use, the interest in effective alternatives to antibiotic growth promoters (AGPs) is increasing. However, little attention is paid to water hygiene, despite water a critical component of the production system. The goal of this study was to assess impacts on broiler performance supplied acidified drinking water using a blend of free and buffered organic acids (OA), in comparison to animals fed in-feed antibiotics. 1360 Cobb 500 cockerels were randomly allocated to one of 5 treatment groups, with 16 replicates of 17 birds/pen. Treatments included: 1) control, 2) BMD (50 g/ton), 3) OA Low (0.5 L/1000L, pH 4.4), 4) OA Medium (1.2 L/1000L, pH 3.8), and 5) OA High (2.0 L/1000L, pH 3.6). The experiment was conducted under commercial conditions, with re-used litter from the previous 3 flocks using spray vaccinated (Coccivac-B52) chicks. Corn-soy based diets were fed in 3 phases: starter crumbles for d 0–10, grower pellets for d 11–22 and finisher pellets for d 23–42. Dosage levels of the drinking water additive were determined based on water titration, with daily adjustments based on pH levels. Body weight (BW) and feed intake (FI) were assessed by pen on d 0, 11, 23 and 42. Footpad scoring was performed on d 42. Pairwise comparison of means (Tukey's) was used to determine significance between treatment means ($P < 0.05$). The observed growth performance was typical of commercial Cobb broilers grown to 42 d with an overall average BW 2.96 kg. Broilers that received OA in the water, regardless of dosage, were observed to have a numerically higher average BW (2.97 kg) compared with the control treatment group (2.92 kg), and similar to the group fed a diet containing BMD ($P = 0.241$). The cumulative FCR (d 0–42) of OA High was significantly lower than control and BMD ($P = 0.021$). Mortality was not significantly different among treatments ($P = 0.266$), although birds given OA-supplemented water had numerically lower mortality rates (−4.4% on average) compared with BMD (7.72%). Footpad quality was significantly improved in OA Medium and OA High compared with control and BMD ($P < 0.001$). The results suggests that the use of free and buffered organic acids in drinking water may be an important component of an integrated management program to support the US broiler industry's move toward antibiotic-free production.

Key Words: organic acids, broiler, antibiotic reduction, performance, footpad quality

252 Performance of broiler chickens fed diets with different nutrient density supplemented with hydroxy copper chloride in combination with a synergistic blend of organic acids. L. Pineda*[2], G. Page[1], N. de Groot[2], and Y. Han[2], *[1]Trouw Nutrition Agresearch, Guelph, ON, Canada, [2]Trouw Nutrition, Amersfoort, the Netherlands.*

An experiment was conducted to evaluate the effects of supplementing hydroxy copper chloride (IBC) in combination with a synergistic blend of organic acids (OA, buffered short– and medium–chain fatty acids, phenolics) on performance, microbiota profile and economics of broiler chickens fed diets with different nutrient density. Cobb broiler chicks (n = 840) were randomly allocated to 5 treatments and provided the following diets: Treatment 1– Standard diet based on Cobb broiler nutrient specification with 15 ppm CuSO4 (Standard), Treatment 2 – modified Cobb broiler diet with low protein and energy content and 15 ppm CuSO4 (Modified), Treatment 3 – As T1 with 50 ppm Zn bacitracin (AGP), Treatment 4 – Standard diet with 150 ppm IBC & synergistic OA in water in combination with a blend of OA in feed (Standard+FA), Treatment 5 – modified diet with 150 ppm IBC + OA (Modified+FA). Birds were reared on floor pens with recycled litter and a house temperature of 32°C from d 5 until d 35. The broilers' body weight and feed consumption were recorded at 14, 28 and 35 d of age; the FCR was calculated as the feed:gain ratio. Digesta sample was collected at d21 for microbiota analysis. Litter moisture and foot-pad score were determined at d21 and 35, while carcass yield was recorded at d35. Data were analyzed by ANOVA with $P \leq 0.05$ considered significant. Birds in all treatments had similar survival rate during the experiment ($P > 0.05$). Feed intake was comparable in all the groups ($P > 0.05$). However, body weight gain was significantly depressed in broilers fed with modified diets compared with AGP and standard diets with or

without FA ($P < 0.05$). The addition of FA in either diets did not result in a significant increase in ADG and production efficiency, but the numerical improvement resulted in parallel performance to broilers fed with AGP supplemented diet. The FCR was lower for broilers fed the standard diet supplemented with FA and AGP compared with the rest of the treatments ($P < 0.05$). The cost per kg gain was lowest for AGP, standard and standard+FA groups in relation to modified diets with and without FA ($P < 0.05$). The effect of treatment on carcass yield, litter moisture and foot-pad score was similar for all groups (all, $P > 0.05$). The supplementation of FA can potentially alter microbiota population, by numerically decreasing the population of pathogenic bacteria (*E. coli* and *Clostridium*) and favoring the proliferation of *Lactobacillus* bacteria ($P > 0.05$). In conclusion, the combined use of blends of organic acids by feed and water and hydroxy copper chloride in standard and modified Cobb broiler diets shows comparable benefits as in-feed antibiotics on performance and health of broilers.

Key Words: broiler chickens, nutrient density, organic acids, hydroxy copper chloride, microbiota

254 Major histocompatibility complex haplotypes and SNP associations in broilers with woody breast. M. Ibrahim*, K. Zozik, and G. Athrey, *Texas A&M University, College Staion, TX.*

The major histocompatibility complex (MHC-B) is a highly conserved, and set of genes that drive the vertebrate adaptive immune system. In chicken, MHC-B haplotypes and (single nucleotide polymorphisms) SNP variants are known to be associated with various disease phenotypes. The woody breast syndrome (WB) is a disorder affecting the broiler breast muscles (Pectoralis major), which is a leading meat quality problem at present. Previous studies on gene expression patterns in WB indicate oxidative stress, and also the upregulation of immune genes, and pathway analyses suggest various systemic disorders and immune disorders. In the current study, our objective was to characterize associations between SNP variants at MHC loci in 2 commercial broiler strains that were positive for WB. For additional context, we used also compared broiler strains against heritage chicken breeds, and the ancestral Red Jungle fowl (RJF). A total of 143 individual birds were genotyped on the custom SNP panel targeting the chicken chromosome 16, initially described in Fulton et al. (2016). The SNP panel examines the 90- SNP loci within and flanking the MHC-B locus spanning between 30,189 to 240,933 bp on chromosome 16. The genotyping was performed at the molecular genetics lab at Hy-Line International. Of the 143 birds genotyped, 97 were from commercial broilers with WB, and the rest were comprised of heritage chicken without a history of WB and RJF. The latter 2 groups served as negative controls. Differences among strains were assessed under the case-control assumption, and each SNP *P*-value was calculated under co-dominant genetic model using pairwise comparisons among the groups. Finally, the genotype data were phased using the program PHASE to identify unique haplotypes within each group. Based on SNP genotyping 50 unique haplotypes were identified in the MHC-B region. Based on the pairwise comparison, between 49 and 62 loci showed a significant association based on *P*-value (<0.01). One particular gene, CD1A2, emerged as being significantly associated across multiple comparisons, suggesting its potential importance. Conclusions: Our study characterized haplotype and SNP diversity across the MHC-B region in broilers with woody breast, as well as other negative control strains. Association tests revealed multiple MHC loci to be of potential importance, while its role in WB etiology needs to be further investigated. The CD1A2 gene, which was found to be significant across multiple comparisons, is known to be involved in non-peptide antigen presentation and deserves further attention.

Key Words: haplotypes, SNP, MHC-B, CD1A2, woody breast

255 Identifying the best housekeeping genes to normalize real-time PCR gene expression data in Pekin ducklings. R. Shanmugasundaram* and M. Lilburn, *OARDC, The Ohio State University, Wooster, OH.*

In real time PCR analysis, gene transcription is normalized to a reference or housekeeping genes to correct for experimental variation, and differences in the quantity of starting material and sample loading. Identifying a correct housekeeping gene with stable transcription across all treatments is important with respect to validating real time PCR data. In the current study, Pekin duck eggs were distributed to 4 treatment groups: Control (37.5°C from embryonic day [ED]1 to 25); Control-LPS (SS-LPS; 37.5°C from ED1 to 25 + LPS at D0); High Temperature + LPS (HH + LPS; 38°C from ED1 to 25+ LPS at D0); Standard-High temperature + LPS (SH-LPS; 37.5°C from ED1 to 10 and 38°C from ED 11 to 25 + LPS at D0). Splenic sample were collected at D1, D3, and D5 post-hatch, mRNA was extracted and cDNA was analyzed for 5 housekeeping genes: Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), β-actin, ribosomal protein S13 (RPS13), ubiquitin, and succinate dehydrogenase. Primers were designed from the duck genome. The stability of the candidate housekeeping genes was analyzed using the Normfinder algorithm. After analysis of the stability values for the 5 housekeeping genes, GAPDH was found to be the most stable reference gene with a stability value of 0.087 followed by β actin (0.088), RPS13 (0.124), SDHA (0.167) and Ubiquitin (0.203). The combination of GAPDH and RPS13 had a stability value of 0.087. GAPDH was identified to be the single best housekeeping gene for which to normalize the real-time PCR data in the Pekin ducklings hatched from thermally manipulated embryos. These results emphasize the importance of validating the potential housekeeping or reference genes before conducting gene expression studies in Pekin ducks.

Key Words: real-time qPCR, reference gene, Pekin ducks, normalizing qPCR, GAPDH

256 Effects of delayed feeding post-hatch on gut barrier-related genes expression during early neonatal development in broiler chickens. M. Proszkowiec-Weglarz*, L. Schreier, K. Miska, S. Kahl, B. Russell, and T. Elsasser, *USDA, ARS, NEA, Beltsville, MD.*

The gastrointestinal tract (GIT) plays a key role in the digestion and absorption of nutrients, but also forms a physical barrier and first line of defense between the host and the luminal environment. A functional GIT barrier (mucus and epithelial cells with tight junctions (TJ)) is essential for optimal health and efficient production in poultry. In the current broiler system, chicks are deprived of food and water up to 72 h due to uneven hatching, hatchery procedures and transportation time to farms. Post-hatch (PH) feed delay results in lower body and organ weight, higher FCR and mortality, and delay PH growth and GIT development. Little is known about the effects of delayed feeding immediately PH on GIT barrier function in chickens. Therefore, the aim of the present study was to characterize gene expression pattern of mucin 2 (MUC2) and TJ related protein genes in small intestine of broiler chickens during early neonatal development and delayed access to feed PH. Newly hatched chicks received feed and water immediately after hatch (FED) or were subjected to 48 h delayed access to feed (NOTFED) to mimic commercial hatchery setting and operations. Birds were sampled (n = 6) at −48, 0, 4, 24, 48, 72, 96, 144, 192, 240, 288 and 336 h PH, and jejunum and distal part of the ileum were collected, cleaned of digesta and snap frozen in liquid nitrogen. The relative mRNA levels of MUC2, fatty acid-binding protein (FABP) 2, occludin (OCLN) zonula occluden 1 (ZO-1), junctional adhesion molecule (JAM) 2 and 3, cloudin (CLDN) 1 and 4 were measured by quantitative PCR and analyzed by 2-way ANOVA. In both tissues, upregulation of MUC2 mRNA was observed in FED during development while FABP2 expression was characterized by an increase during the first 24 h PH followed by decrease afterward ($P < 0.05$). OCLN mRNA expression was downregulated ($P < 0.05$) during development in jejunum and ileum of FED birds. Expression of TJ proteins ZO-1, JAM2 and 3 decreased in early phase of PH development (from −48 to 48–72 h) in jejunum and ileum of FED birds. Similar expression pattern was observed for CLDN1 and 4 in both tissues. Delayed feeding (NOTFED) for first 48 of PH development significantly ($P < 0.05$) affected the mRNA expression level of MUC2, OCLN, FABP2, ZO-1 and JAM2 in jejunum, and MUC2, OCLN, ZO-1, JAM2, JAM3 and CLDN4 in ileum. In general, MUC2 expression was downregulated while, OCLN, FABP2, ZO1, JAM2 and JAM3 were upregulated between 24 and 72 h in NOTFED in comparison to FED. These results indicate that delayed access to feed PH may affect the structure and/or function of TJ and therefore the GIT barrier function.

Key Words: tight junction, gut, development, delayed feeding, chicken

257 Protective effects of synbiotic supplementation in *Eimeria* challenged turkey poults. C. Pender*[1], G. R. Murugesan[1], B. Syed[2], W. Briggs[3], M. Lilburn[3], and L. Bielke[3], *[1]Biomin America Inc., Overland Park, KS, [2]Biomin Holding GmbH, Getzersdorf, Austria, [3]Ohio State University, Wooster, OH.*

As consumer and regulatory opinions evolve, the poultry industry is actively seeking alternative solutions to reduce usage of antibiotics and chemicals. As research develops, probiotics have been receiving increased attention for their ability to improve enteric health in poultry. The objective of this study was to evaluate the effects of synbiotic (combination of probiotics and prebiotics) supplementation on performance and intestinal health of turkey poults during a mixed *Eimeria* challenge. A total of 1,080 d-old male poults were provided either a standard diet, a diet supplemented with coccidiostat (Clinacox) or a diet supplemented with synbiotic (PoultryStar me). On d 16, half of the pens were administered a mixed *Eimeria* challenge (*Eimeria adenoeides* and *Eimeria meleagrimitis*) via oral gavage resulting in a total of 6 groups. Each group consisted of 6 replicate pens with 30 birds per pen. The trial was conducted over a 45 d period with performance measurements recorded on d 0, 16, 21, 35, and 45. On d 21 and 28, 5 birds per pen were euthanized for intestinal lesion scoring. Fresh feces was also collected daily starting on d 21 to determine oocyst shedding. Data were analyzed as an ANOVA with significance reported at $P \leq 0.05$. Challenged birds supplemented with synbiotic showed significantly improved body weight throughout the trial when compared with the challenged control and was similar to the coccidiostat group. Body weight gain and FCR was also improved in challenged birds administered synbiotic or coccidiostat from d 21–28. On d 21, the percentage of birds displaying lesions in the jejunum and ileum was significantly reduced in birds provided synbiotic or coccidiostat. Similar to non-challenged controls, fecal samples from challenged birds supplemented with either synbiotic or coccidiostat displayed no evidence of oocyst shedding. Overall, these results suggest supplementation of poultry-specific synbiotic may be a viable solution to alleviate negative consequences observed during a coccidiosis challenge in turkeys.

Key Words: coccidiosis, probiotic, prebiotic, oocyst shedding, lesions

258 Performance and lesions of turkeys fed diets supplemented with bastion and challenged with coccidia. N. Evans*[1], J. Schrader[2], F. Hoerr[2], P. Karnezos[1], R. Dvorak[1], C. Novak[3], and M. Masadeh[3], *[1]PMI Nutiritional Additives, Harrisonburg, VA, [2]Veterinary Diagnostic Pathology, LLC, Fort Valley, VA, [3]Purina Animal Nutrition, Shoreview, MN.*

A 133-d study was conducted to compare performance, mortality, and lesion scores of tom turkeys (10–11 replicates, 82 birds/pen) supplemented with Bastion (a proprietary blend of phytogenics, prebiotics, and probiotics) and challenged with 2 species of coccidia. Experimental treatment groups were: 1) positive control (challenge + no additives); 2) Coban (challenge + Coban 54 g/ton 0–35 d, 63 g/ton 36–56 d, 54 g/ton 57–90 d); and 3) Bastion (challenge + Bastion 1.5 lb./US ton 0–35 d, 1 lb./US ton 36–133 d). Birds were challenged with *Eimeria meleagrimitis* (5,000 oocyst/bird) and *Eimeria adenoides* (5,000 oocyst/bird) at age 21 d. Pen weights, feed intakes, and mortality were collected throughout the study and evaluated at age 42 and 133 d. To coincide with cocci cycling, oocyst per gram of fecal material (OPG) was evaluated at 12 and 18 d post-challenge (age 33 and 39 d, respectively) and lesion scores were evaluated at 11 d post-challenge (age 32 d). Performance and OPG data were analyzed by ANOVA and when the model was significant ($P \leq 0.05$), a post-hoc analysis (LS Means Student's *t*-test) was performed. Lesion scores were analyzed by either Kruskal-Wallis or Fisher's Exact (Freeman-Halton) test. At age 42 d, body weight and feed conversion were significantly improved in the Coban treatment group ($P \leq 0.05$) and numerically improved in the Bastion treatment group (trending toward significance: $P \leq 0.10$) when compared with the positive control treatment group. Enteritis and cocci oocyst shedding were noted in all treatment groups; however, no significant differences were observed between treatment groups. Interestingly, the number of birds with microscopic coccidial lesion scores ≥2 was significantly lower in both the Coban and Bastion treatment groups ($P \leq 0.05$) when compared with the positive control treatment group. No significant differences in performance were observed between treatment groups at age 133 d. In this study, Bastion appears to improve early performance and reduce microscopic coccidial lesions in challenged tom turkeys.

Key Words: coccidia, turkey, performance, mortality, coccidial lesions

259 Role of various predisposing factors in experimental induction of necrotic enteritis in broiler chickens. G. Saleem*[1], J. Houdjik[2], and N. Sparks[2], *[1]University of Veterinary and Animal Sciences, Lahore, Lahore, Punjab, Pakistan, [2]SRUC, Edinburgh, United Kingdom.*

Necrotic enteritis (NE) is an important enteric disease in poultry production. The primary etiological agent of disease is *Clostridium perfringens* type A however numerous additional influential factors mainly diet, co-infection with other pathogens particularly coccidia, as well as environmental and management factors has also been reported as predisposing factors. Here, a series of experiments were conducted to investigate the importance of the various potential predisposing factors, in isolation and in combination, that are believed to play a role in sub-clinical NE development: feed withdrawal, dietary protein sources, co- infection with coccidia, *C. perfringens* dose and contact with reused litter. In an experiment feed withdrawal up to 24 h in experimentally challenged birds did not result in NE specific lesions. Replacing dietary soyabean meal with potato protein concentrate or canola meal or adding synthetic trypsin inhibitor to the soyabean meal control diet did not induce sub-clinical NE in birds housed on reused litter, a natural source of *C. perfringens* challenge. An in vitro *experiment* showed that growth of *C. perfringens* on in vitro digested grower diets was prolonged following the addition of fishmeal, suggesting that the role of fish meal as a predisposing factor for in vivo sub-clinical NE cannot be excluded. All subsequent diets therefore contained high levels of fish meal. When this was used in combination with high dose of coccidial vaccine, a repeated in-feed challenge for 3 d at 10^2 colony forming units (cfu) *C. perfringens* per g feed did not result in sub-clinical NE, though at 10^9 cfu/g resulted in 10% of challenged birds (3 out of 30) showing NE-specific lesions. The failure to significantly induce sub-clinical NE in present experiments suggests that challenging the birds with *C. perfringens* in the presence of one or 2 suspected predisposing factors may not provide a suitable experimental model. Indeed, when birds were dosed twice daily with 10^8 cfu *C. perfringens* for 3 d in the presence of high levels of fishmeal, canola meal as main protein source, coccidial and IBD vaccinations, and feed withdrawal before challenge, 40.6% of the challenged birds developed lesions of sub-clinical NE without inducing mortality. This concurred with reduced growth performance relative to

the sham-infected control birds, and thus is a successful model for induction of sub-clinical NE. Improved knowledge of the effect of different dietary components on the growth of C. *perfringens* may help in the formulation of broiler diets to assist in further reducing the incidence of NE particularly in the absence of antimicrobial growth promoters.

Key Words: necrotic enteritis, experimental induction, predisposing factors, Clostridium perfringens

260 Probiotic, 1-monglycerides, and feed acidification improve performance and reduce necrotic enteritis when fed to broiler chickens challenged with *Clostridium perfringens.* G. Mathis*[1], B. Lumpkins[1], P. Ader[2], M. Coelho[4], and C. Hofacre[3], *[1]Southern Poultry Research Inc., Athens, GA, [2]BASF SE, ENS/LD, Lampertheim, Germany, [3]Southern Poultry Research Group, Watkinsville, GA, [4]BASF, Florham Park, NJ.*

Clostridium perfringens-induced necrotic enteritis (NE) has become a great concern to the poultry industry. A 28 d cage broiler chicken study consisting of 8 treatments and 8 replications examined the reduction of NE by feeding no additive (non-challenged (NM) and (challenged (NMI)), SILOhealth 104 (SH) a 1-monglycerides; 2.5 and 5.0 kg/mt, MIYA-GOLD (MG) a *Clostridum butyricum* probiotic, 0.5 and 1.0 kg/mt, Amasil NA (AN), sodium formate, 8.0 and 12.0 kg/mt, or BMD, antibiotic, 50 g/t. On D14, all broilers were orally inoculated with a low dose of *E. maxima*. On D19, 20, and 21, all birds, except NM, were orally dosed with *C. perfringens.* Birds and feed were weighed on D0, 14, 21, and 28. On D21, 3 birds per cage were NE lesion scored (0–3). The NE model was successful in producing a severe infection with 50% reduction in weight gain, 46 points higher FCR, and 31% NE mortality in the NMI birds. Challenged birds fed either level of the MG, SH, or AN had significantly better weight gain (D0–28), lower NE lesion scores and % NE related mortality compared with NMI. FCR was significantly improved with the higher dose level of each feed additive and was not different from the BMD fed birds. The antibiotic BMD improved the performance (FCR and weight gain) and reduced NE lesions and NE mortality (0.9 and 6% respectively). These results emphasize the benefits of either feeding SILOhealth, MIYA-GOLD, Amasil NA, or BMD by reducing the negative impact of NE in broiler chickens.

Key Words: clostridium, coccidiosis, acidifier, probiotic, mongleyceride

261 Live *Salmonella typhimurium* vaccination as a field intervention tool for reducing salmonella prevalence and load at the processing plant. M. Da Costa*, K. Cookson, and J. Schaeffer, *Zoetis, U.S. Poultry, Durham, NC.*

The Food Safety and Inspection Service's (FSIS) *Salmonella* spp. compliance guidelines for the poultry industry have progressively become more stringent. Taking a further step in salmonella control, FSIS recently started publishing processing plant salmonella categories. Consequently, there is an urge to reduce salmonella prevalence and load coming into the processing plant. One available interventional strategy is live *Salmonella typhimurium* (ST) vaccination. The objective of this study was to evaluate the effect of live ST vaccination on salmonella prevalence, load and serogroup distribution on carcass rinsates at hot rehang. There were 2 field trials conducted in a 2-week on/off fashion of Poulvac ST vaccinated birds. The first trial was run in a conventional broiler complex for a length of 14 weeks, whereas the second trial was run in an organic broiler complex for a length of 13 weeks. Each trial had a total of 6 weeks of Poulvac ST vaccination. Sampling consisted of 3 carcass rinsates per flock for salmonella enumeration (MPN testing capped at 40), prevalence (enrichment samples only done for organic trial) and serogrouping. The data analysis consisted of salmonella percent positives, geometric mean counts, percent super shedders (MPN > 40), serogroup distribution and flock performance. Since there was not a true randomization of the treatments (intermittent blocks of 2 weeks) only descriptive statistics could be performed. Poulvac ST vaccinated conventional broiler flocks were 68% less positive at the plant than controls (26.8% vs 8.7%). Vaccination of conventional broilers also reduced salmonella counts in half (2.02 vs. 4.61) at the plant when compared with controls. Of the 33 positive control samples, 8 were from serogroup B, 5 from C1, and 17 from C3. Of the 9 positive samples from Poulvac ST, 7 were C3 and 2 E1. In the organic broiler trial, there was a reduction of 31.7% positive carcasses at the plant related to vaccine intervention. There was also a decrease in salmonella load (4.31 vs. 3.53) and percent super shedders (5.44% vs. 4.41%) in Poulvac ST flocks. Of 68 positive samples from the controls, 25 were from serogroup B and 40 from C. Of the 43 positive samples from Poulvac ST, 12 were B and 27 C. In addition to salmonella reductions, an overall improvement in adjusted feed conversion was observed in Poulvac ST vaccinated broilers. In conclusion, Poulvac ST reduced salmonella prevalence and load on broiler carcasses at hot rehang, with a concurrent displacement of salmonella serogroups and improvements in bird performance.

Key Words: *Salmonella*, vaccine, broilers, serogroups, food safety

262 Non-Mhc background genes reduce Rous sarcoma progression in major histocompatibility (B) complex genotype *B5B5*. R. Taylor*[1] and R. Kopulos[2], *[1]West Virginia University, Morgantown, WV, [2]Northern Illinois University, DeKalb, IL.*

Tumor growth induced by Rous sarcoma virus (RSV) exhibits genetic control. The principal genes influencing tumor growth belong to the major histocompatibility (B) complex but multiple reports document effects of other genes as well. Tumor outcome, either regression or progression, has been characterized in various Mhc haplotypes. The study's objective was to examine RSV tumor growth in the progressive *B5B5* genotype in 2 different genetic backgrounds. Congenic Line 6.15-*5* (*B5B5*) sires were crossed to dams from 2 lines: TK (*B*15*B*21) and the Arkansas Regressor (AR) Line (13, 221 and 221–2 haplotypes segregating). The F_1 progeny were backcrossed to their respective dam line followed by *inter se* mating to produce *B5B5* progeny designated 5TK or 5AR. A 5TK sire was mated to multiple 5AR dams to produce 5TK5AR chicks whereas the reciprocal cross mated a 5AR sire to multiple 5TK dams to produce 5AR5TK chicks. Both groups had a genetic composition of 25% Line 6.15-*5*, 37.5% Line TK and 37.5% Line AR with the *B5B5* genotype, but different sources of the *Z* chromosome and mitochondrial genes. In 3 separate trials, 6 wk old 5TK5AR and 5AR5TK chicks were wingweb injected with 20 pfu of a Bryan high-titer RSV subgroup A pseudotype. Tumor size was evaluated 6 times during a 10 wk period after inoculation using a defined scoring system. A tumor profile index (TPI) for each bird was compiled using the 6 tumor scores over the 70 d experimental period. Mean tumor size within a time period and mean TPI were compared by ANOVA. Tumors size increased throughout the study in 5TK5AR (n = 22) progeny. In contrast, tumor growth plateaued at 6 wk post-inoculation in 5AR5TK (n = 30) birds. Tumor size scores differed significantly at 4, 6, 8 and 10 wk post-inoculation. Complete tumor regression was not observed in 5TK5AR birds but was evident in 23.3% (7/30) of 5AR5TK individuals. The higher TPI of 5TK5AR birds (3.0 ± 0.1) compared with that found in 5AR5TK birds (2.2 ± 0.2) differed significantly. In conclusion, the distinct tumor growth in 2 Mhc identical *B5B5* groups suggests non-Mhc genes influencing tumor growth. The genetic composition

of the 2 groups was the same but individual genes as well as the *Z* chromosome and mitochondrial genes are different. Non-Mhc genes in the 5AR5TK birds may have been overcome the documented cross reactivity between the B5 antigen and one or more RSV antigens that lowers the antitumor response.

Key Words: MHC, Rous sarcoma virus, oncogene, tumor, progression

263 Evaluation of antimicrobial action of chitosan and acetic acid on broiler cecal bacterial profiles in anaerobic cultures inoculated with *Salmonella* Typhimurium. M. U. Sohail*, *Qatar University, Doha, Qatar.*

The chitosan is known to have antimicrobial properties against a range of microbes. Population effects of chitosan preparations were examined in broiler cecal bacterial anaerobic cultures inoculated with *Salmonella* Typhimurium. Three different molecular weight (MW) chitosan preparations were used: low (LMW), medium (MMW), and coarse (CMW), using acetic acid (AA) as carrier. Broiler cecal contents were inoculated with 10^5 cfu of *S.* Typhimurium and cultured anaerobically at 40°C for 24 h in 5 treatment groups: *S.* Typhimurium and cecal contents control (CON), AA plus *S.* Typhimurium and cecal contents (AA), LMW-treated *S.* Typhimurium and cecal contents, MMW-treated *S.* Typhimurium and cecal contents, and CMW-treated *S.* Typhimurium and cecal contents. The population effects of chitosan preparations on cecal bacteria and *S.* Typhimurium were assessed by 16S rRNA Illumina MiSeq sequencing. Sequencing revealed zero prevalence of *Salmonella* spp. in the AA and the CMW groups. The AA and CMW groups also had lower bacterial diversity and species richness (α diversity index; chao1, Shannon, and observed species). However, unweighted Unifrac Beta diversity based PCoA plots revealed no significant differences in microbial diversity among the different treatment groups. Kruskal-Wallis test analysis of treatment effects on taxonomic distribution of bacteria revealed no differences ($P > 0.05$) in the percentages of bacteria among the different groups. In conclusion, culturing the cecal contents in the presence of AA and CMW treatments suppressed bacterial growth and within sample bacterial diversity. Both LMW and MMW chitosan preparations had no effect on either *S.* Typhimurium or bacterial growth and diversity.

Key Words: chitosan, *Salmonella*, in vitro culture, cecal microbiome, 16S rRNA sequencing

264 Colonization of internal organs by *Salmonella* Enteritidis in experimentally infected laying hens of four commercial genetic lines in conventional cage and enriched colony housing. R. Gast*[1], P. Regmi[2], R. Guraya[1], D. Jones[1], K. Anderson[3], and D. Karcher[2], *[1]USDA-ARS, Athens, GA, [2]Purdue University, West Lafayette, IN, [3]North Carolina State University, Raleigh, NC.*

Human infections with *Salmonella* Enteritidis are often attributed to the consumption of contaminated eggs, so the prevalence of this pathogen in commercial egg-laying flocks is a significant public health concern. Internal contamination of the edible contents of eggs results from bacterial colonization of reproductive tissues in systemically infected hens. Environmental conditions can exert powerful influences on the progress of avian *Salmonella* infections, but the food safety consequences of different housing systems for egg-laying hens remain incompletely understood. The present study assessed the invasion of internal organs by *S.* Enteritidis in groups of experimentally infected laying hens of 4 commercial genetic lines (designated as white egg lines W1 and W2 and brown egg lines B1 and B2). In 2 trials, groups of hens from each line were housed at a stocking density of 555 cm^2 of floor space per bird in both conventional cages and colony units enriched with access to perches and nesting areas. All hens were orally inoculated with a dose of 5.75×10^7 cfu of a mixture of 2 strains of *S.* Enteritidis. At 6–7 d post-inoculation in each trial, hens were euthanized and samples of 5 internal tissues were removed for bacteriologic culturing. The overall frequency of recovery of *S.* Enteritidis was significantly greater ($P < 0.05$ in Fisher's exact test) for intestinal samples from the 2 white egg lines combined than from the 2 brown egg lines combined in both conventional cage (72.2% vs. 50.0%) and enriched colony housing systems (66.7% vs. 37.5%). The frequency of intestinal isolation of *S.* Enteritidis from line B1 was significantly higher from hens in conventional cages (47.2%) than from hens in enriched colonies (22.2%), but no corresponding differences were observed for the other 3 hen lines. There were no significant differences between the 4 lines of hens or between the 2 housing systems in the frequency of isolation of *S.* Enteritidis from livers, spleens, ovaries, or oviducts. These results demonstrate that *S.* Enteritidis colonization can vary between genetic lines of egg-laying hens and that some lines are also subject to significant housing system influences on *Salmonella* susceptibility.

Key Words: *Salmonella* Enteritidis, chicken, internal organ, genetic line, housing system

265 The effects of refined functional carbohydrates supplemented to layer pullets on environmental *Salmonella* prevalence under commercial conditions. J. Nezworski*[1], D. Karunakaran[2], and S. Jalukar[2], *[1]Blue House Veterinary LLC, Buffalo Lake, MN, [2]Church and Dwight, Princeton, NJ.*

Enzymatic hydrolysis of yeast produces refined functional carbohydrates (RFC) which can have activity against gram-negative bacteria. This study was conducted to evaluate the effect of RFC on salmonella loads in egg type chickens. A total of 4 commercial flocks were selected and assigned to 2 treatments. Day-old White Leghorn type layer chicks were fed a non-medicated commercial pullet diet in mash form supplemented with CELMANAX (Arm and Hammer Animal Nutrition, Princeton, NJ), 0 or 500 g/MT. Each commercial pullet flock consisted of approximately 90,000 birds housed in stacked deck belted cage systems. *Salmonella* sampling was done on chick papers and from environmental samples taken at Pre-fill, 10 weeks, and 16 weeks. Samples were collected using NPIP and FDA environmental sampling procedures and cultured as per NPIP culture methods. Environmental samples were collected at a rate of 2 swabs per row resulting in 8–14 samples per time point. The chick papers were collected at a rate of 10% of the papers per breeder flock resulting in 5–14 samples per time point. Qualitative and quantitative *Salmonella* testing was done by counting the number of presumptive *Salmonella* colonies on the XLT plates. Samples that had too numerous to count (TNTC) colonies were given a numeric value of 100 for evaluation purposes. The qualitative and quantitative results from 2 control and 2 RFC supplemented farms were averaged. Pre-fill sampling isolated *Salmonella* from both barns housing RFC fed pullets and one barn housing control fed pullets. Chick papers were positive for one control assigned flock and negative for the second control assigned flock and both RFC assigned flocks. RFC barns were 16% and 23% positive for presumptive *Salmonella* at 10 and 16 weeks compared with 29% and 54% of control barns. Fewer TNTC *Salmonella* samples were isolated from barns housing RFC pullets (4% and 16%) compared with control barns (9 and 30%) at 10 and 16 weeks. Furthermore, of the samples which could be enumerated, similar *Salmonella* counts were isolated from both treatments at 10 weeks and 4 cfu/g was isolated at 16 weeks from RFC pullets and 13 cfu/g from control pullets. These data demonstrated that RFCs were unable to eliminate *Salmonella* under commercial pullet rearing conditions but could reduce presumptive *Salmonella* prevalence and counts.

Key Words: layer, *Salmonella*, yeast, egg, food safety

266 The effectiveness of a feed-grade sodium formate in feed or drinking water in reducing *Salmonella* Heidelberg colonization of broilers. J. Jendza*[1], C. Hofacre[2], and R. Berghaus[3], *[1]BASF Corp., Florham Park, NJ, [2]Southern Poultry Research Group, Watkinsville, GA, [3]The University of Georgia, Athens, GA.*

A report of meat processing plants by the USDA-FSIS and FDA NARMS 2002–2012 found *Salmonella* Kentucky, *S. enteritidis* and *S.* Heidelberg had the highest prevalences in poultry meat birds. *S. enteritidis* and *S.* Heidelberg were the 2 most commonly associated with human illness associated with poultry. A study was conducted to evaluate the effectiveness of a feed grade sodium formate (formate) product (0.60%) fed continuously form d 1 to 42 or d 35 to 42 or in the birds drinking water d 37 to 42 versus a sodium bisulfate (bisulfate) product in the drinking water d 37 to 42. There were 8 replicate floor pens per treatment with ad libitum feed and water. All treatments had one-half of chicks in each pen challenged and tagged at 1 d of age with a nalidixic acid resistant *S.* Heidelberg. A typical 10 h feed withdrawal before slaughter was performed and at 42 d 5 ceca of challenged (direct) and 10 ceca of penmates (horizontal challenged) were aseptically removed. *Salmonella* prevalence and enumeration were performed with tetrathionate plus iodine (41.5°C) then XLT-4 plates (37°C) with 25mg/ml nalidixic acid. Enumeration was performed by micro most probable number (MPN) method of Berghaus et al., 2013. Crop pH was measured on the first 5 horizontal challenged birds necropsied. There were no significant differences in means of crop pH (range 6.19 to 5.67; all sodium formate treatments resulted in a pH below 6.00). The continuous fed formate had a significant reduction in ceca S.H. prevalence (46.7%^{a}), followed by feed formate at 35–42 (49.2%^{ab}), water formate (62.5%^{ab}) and water bisulfate (75%^{b}). All of the formate treatments in the feed had a numerical reduction in S.H. MPN/g with the d1–42 having the greatest reduction. Using a Tobit regression model to more closely estimate the ceca *Salmonella* MPN status (censoring culture negative at $-0.5\log_{10}$ MPN/g) demonstrated that continuous fed formate significantly lowered the estimated mean $\log_{10}$ MPN/g (-0.79^{a}), followed by d35–42 formate (-0.69^{ab}), water formate (-0.15^{ab}) and water bisulfate (0.11^{b}). In conclusion, use of the feed grade sodium formate in the feed or drinking water of broilers can significantly decrease the prevalence and number of S.H. positive ceca.

Key Words: broiler, feed-grade sodium formate, *Salmonella* Heidelberg, *Salmonella* prevalence, *Salmonella* number

267 Microbiological assessment of poultry feed treated in a feed conditioner with steam. G. Morantes*, E. Margas, and B. Conde-Petit, *Buhler Group Inc., Plymouth, MN.*

A study was carried out to characterize the inactivation kinetics of *Salmonella* spp. in animal feed treated with saturated steam as part of the pelleting process applied in a conditioning system for feed. The target strain of *Salmonella* was selected by first identifying the most appropriate strains of *Salmonella* commonly associated with feeds and feed materials through a review of published literature. *Salmonella* strains were then screened within a custom-fabricated steam conditioner test rig constructed to simulate the mixer portion of the conditioning system in which the product is mixed with saturated steam. Inoculated feed was exposed to steam heating at 85°C, allowing selection of *Salmonella* Agona as the most resistant serotype. Further experiments in which triplicate strains of this serotype were screened allowed selection of *Salmonella* Agona RA1052, isolated from cotton seed, as the most resistant strain to be used in determination of D- and z-values at selected moisture levels in poultry feed. Results showed that D-values for *S.* Agona strain RA1052 in poultry feed mash adjusted to 12% moisture, determined within a customized autoclave, were 178.2, 63.0, 19.2, 10.2 and 3.1 s at 65, 70, 75, 80 and 85°C, respectively. The z-values were calculated at 12% and 18% moisture. At 18% moisture we found a 2–3 fold decrease in heat resistance, when compared with the 12% moisture level. In addition, comparative D-values were determined in poultry feed mash for the surrogate organism *Enterococcus faecium* ATCC 8459 (NRRL B-2354) at 70°C. D-values were calculated at 12% and 18% moisture respectively, and compared with the ones obtained with *S.* Agona. The results confirm that the heat resistance of this organism is equivalent to the most resistant *Salmonella* strain in poultry feed, allowing its use as a surrogate in on-site challenge tests of feed conditioning processes applied to feeds.

Key Words: microbiological, feed conditioner, *Salmonella* strains, surrogate, poultry feed

268 The quantification of viable *E. coli* as a poultry processing indicator organism by flow cytometry. K. Feye* and S. Ricke, *University of Arkansas, Fayetteville, AR.*

Economic pressure to increase poultry processing line speed leading to the potential implementation of automation. However for this to occur will require profound innovation in food safety and microbial monitoring systems for effectively conducting microbial screening under these conditions. Rinsates are commonly collected to evaluate poultry contamination and microbial load throughout the processing process. By utilizing flow cytometry (FC) to analyze rinsates, the microbial detection can occur within hours versus standard microbial and molecular methods, which require as much as 8 to 24 h. By doing so, poultry products can be monitored much more frequently within the daily processing cycle. Accordingly, it is essential to initially develop tools that can accommodate this task both in the lab and field. Non-pathogenic indicator organisms have been used effectively and safely to predict foodborne pathogen dissemination patterns in processing plants. Thus, the objective of this study was to determine the ability of FC to quantitate and isolate live bacteria from rinsates. Non-avian Top10 *E. coli* were grown in static cultures overnight in Mueller Hinton broth. Cultures were divided into one of 2 50 mL conical tubes and pelleted at 10,000 g at 4°C for 10 min. Cultures were either vortexed and re-suspended in 2 mL 100% ethanol or 0.85% sodium chloride, then incubated for 45 min at room temperature. Live and dead cultures were stained using the Molecular Probes BacLight Bacterial Viability and Quantitation Kit (Thermofischer) with either 10 mM of SYTO 9 bacteria stain and/or 60 mM of propidium iodide live/dead stain. Absolute quantitation was evaluated via Molecular Probes Microsphere standards, which are 6.0 μm diameter microspheres at a concentration of 1.0 × 10e8 beads/mL. Using the 18-h culture, the live/dead standard was evaluated via FC (% Live: % Dead: 100:100, 75: 25, 50:50, 25:75, 0:100) to verify detection and sensitivity of the live/dead cocktails at different dye concentrations. SYTO9 dye stained best at 10 mM of dye. Propidium iodide stained best at 60 mM, with no improvements at greater concentrations. Autofluorescence was observed in the Propidium Iodide channel. The incorporation of fully live, 50:50 live:dead, and 100% dead controls were employed. Data indicates that it is possible to identify and sort live indicator organisms, such as *E. coli*.

Key Words: indicator organism detection, microbiological quantitation, flow cytometry, *E. coli*, poultry processing

269 Identifying turkey-specific *Lactobacillus* strains that inhibit poultry pathogens, and exploring prebiotic-probiotic combinations that enhance their growth. J. V. Thomas*, B. Weber, A. Wakil, and T. Johnson, *University of Minnesota, Saint Paul, MN.*

Alternatives to antibiotics are urgently needed in poultry production, as the antibiotic use is being reduced and bacterial pathogens are increasingly resistant to antibiotics used for disease treatment. *Lactobacillus* spp is known for their ability to competitively exclude or inhibit pathogen growth in the host, and their ability to enhance performance. Furthermore, combining a prebiotic that specifically feeds the probiotic may better ensure its success in the host. Previous work has identified turkey-specific lactobacilli that enhance poult performance. In the present study, we hypothesized that prebiotics may selectively enhance the growth of these strains, and that their cell-free culture supernatants (CFCS) may inhibit the growth of common poultry pathogens. Two experiments were conducted to determine the effect of lactose and mannan oligosaccharide (MOS) as prebiotics promoting the growth of turkey-source *Lactobacillus* strains. For prebiotic-probiotic experiments, overnight cultures of different strains of *L. johnsonii* and *L. aviarius* were inoculated (10^5 cfu/mL) into minimal media alone or with glucose (positive control) or different concentrations of lactose and MOS. Bacterial enumeration was performed at 0hr, 4hr and, 8hr. For pathogen inhibition experiments, CFCS of the same strains were tested against different serotypes of *Salmonella* and *Escherichia coli* using various percentages of CFCS in pH-adjusted or non-adjusted broths. Optical densities were measured throughout growth. We identified differences in the apparent utilization of lactose and MOS between bacterial species, and between strains within species. Similar differences were observed for the ability of species and strains to inhibit bacterial pathogens. These results indicate that the growth of *Lactobacillus* can be enhanced using a custom prebiotic approach, but that strain selection is critical to identify those that colonize, utilize the correct prebiotic, and inhibit the desired pathogens.

Key Words: *Lactobacillus* spp., prebiotics, cell-free culture supernatant, alternative to antibiotics, pathogen

270 Organic acids and nature-identical compounds increase the susceptibility of *E. coli* and *Salmonella* Enteritidis to broad-spectrum antibiotics. E. Grilli*[1], A. Toschi[1], B. Tugnoli[2], B. Rossi[2], and A. Piva[1], [1]*University of Bologna, Ozzano Emilia, Italy,* [2]*Vetagro, Reggio Emilia, Italy.*

Aim of this study was to assess the ability of nature-identical compounds (NIC) or organic acids (OA) to increase the sensitivity of *E. coli* and *S.* Enteritidis to broad spectrum antibiotics (AB). For this purpose the minimal inhibitory concentration (MIC) of amoxicillin (AMOX) and neomycin (NEO) was tested against one strain of *E. coli* and one strain of *S.* Enteritidis*,* both isolated from broilers liver, alone or in combination with sorbic acid (S), benzoic acid (B), thymol (T), or carvacrol (C). The antimicrobial activity was evaluated with micro-dilution method in Brain Heart Infusion broth (BHI) in which the substances were dissolved at the following concentrations: AMOX and NEO at 128, 64, 32 mg/L for *E. coli*; AMOX at 1, 0.5, 0.25 mg/L and NEO at 128, 64, 32 mg/L for *S.* Enteritidis; S and B at 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 mM; T and C at 3.75, 1.87, 0.94, 0.47, 0.23, 0.12 and 0.06 mM. Control strains were incubated with BHI only. The analysis was performed on 96-wells microtiter plates with 10^5 cfu/mL. The plates were incubated for 24 h at 37°C. After incubation, the growth inhibition was evaluated by absorbance measurement at 630 nm. Data were analyzed with one-way ANOVA and differences were considered significant at $P < 0.05$. *E. coli* was resistant to both antibiotics, whereas C and T MIC was 1.87 and 3.75 mM, respectively, and S and B was 50 mM. The addition of 50% of the MIC dose of both C and T to the lowest dose of both AB allowed to completely inhibit *E.coli* growth ($P < 0.05$). Again, the addition of 50% of the MIC dose of B and S to AMOX and NEO allowed to reduce the growth of *E. coli* by 92% and 76%, and 82% and 56%, respectively ($P < 0.05$). *S.* Enteritidis was sensitive to the highest dose of AMOX (1 mg/L) and resistant to NEO up to 128 mg/L. The MIC of C and T was 1.87 and the MIC for B and S was 50 mM. Adding 0.94 mM (50% of the MIC) of either C or T to the lowest dose of either AB allowed to completely inhibit the growth ($P < 0.05$). Similarly, adding 12.5 mM (25% of the MIC) of B or S to the lowest dose of AMOX and NEO allowed to decrease the growth of *Salmonella* by 80% and 100%, respectively ($P < 0.05$). In conclusion, *E. coli* and *S.* Enteritidis susceptibility to amoxicillin and neomycin was significantly increased by either OA or NIC. As these substances are commonly used as feed additives, this opens new perspectives to the problem of loss of efficacy of broad-spectrum antibiotics and antibiotic resistance.

Key Words: antibiotics, organic acids, nature-identical compounds, *Salmonella* Enteritidis, *E. coli*

271 Effects of *Propionibacterium freudenreichii* probiotic, mannan oligosaccharide prebiotic, and vaccination on multidrug-resistant *Salmonella* Heidelberg in 7-week-old commercial turkeys. D. V. T. Nair*, J. V. Thomas, G. Dewi, S. Noll, and A. K. Johny, *University of Minnesota, Saint Paul, MN.*

Salmonella enterica serovar Heidelberg (SH) colonizes the cecum of turkeys and can potentially contaminate the carcasses due to faulty evisceration during processing. The objective of this study was to evaluate the effects of an allochthonous probiotic bacterium, *Propionibacterium freudenreichii* (PRO), a mannan oligosaccharide prebiotic (PRE), and a *Salmonella*-specific vaccine (VAC) against multidrug-resistant (MDR) SH in 7-week old commercial turkeys. The treatment groups (8 turkeys/group; n = 72 turkeys/experiment; 2 experiments) in the study were: negative control (-SH, -PRO, -PRE, -VAC), SH control (+SH, -PRO, -PRE, -VAC), PRO group (+SH, +PRO, -PRE, -VAC), PRE group (+SH, -PRO, +PRE, -VAC), VAC group (+SH, -PRO, -PRE, +VAC), PRO+PRE group (+SH, +PRO, +PRE, -VAC), PRO+VAC group (+SH, +PRO, -PRE, +VAC), PRE+VAC group (+SH, -PRO, +PRE, +VAC) and PRO+PRE+VAC group (+SH, +PRO, +PRE, +VAC). VAC was applied as a coarse spray on d 1 followed by a revaccination at 3-weeks of age through drinking water to the turkeys. PRO (10^{10} cfu/ml) was supplemented through drinking water whereas PRE (0.2%) was added to the feed for 7-weeks to the turkeys. Turkeys were challenged with a 2011 ground turkey outbreak strain of SH (10^6 cfu/turkey) at wk 6. The birds were euthanized at 2- and 7 d post-inoculation (PI) and SH colonization and its dissemination to liver and spleen were determined. The PRO, PRE, and VAC treatments resulted in 1.0-, 1.5-, and 2.0- $\log_{10}$ cfu/g reduction in the cecal colonization of SH, respectively, compared with the SH control at 2 d PI ($P < 0.05$), whereas the combination of the 3 interventions resulted in an average 2.3 $\log_{10}$ cfu/g reduction ($P < 0.05$). At 7 d PI, the VAC treatments were highly effective resulting in the complete reduction (enrichment negative) of SH when applied alone or in combination ($P < 0.05$). In addition, VAC treatments completely inhibited the dissemination of SH to liver and spleen ($P < 0.05$). The results revealed that the combination of *Propionibacterium freudenreichii* probiotic, mannan oligosaccharide prebiotic, and *Salmonella* vaccination could be an effective strategy to control MDR SH in commercial turkeys (MIN-16–102).

Key Words: *Salmonella* Heidelberg, *Propionibacterium freudenreichii*, mannan oligosaccharide, vaccination, antibiotic alternative

272 A temporal analysis of mycotoxin occurrence in fresh US corn from 2013 to 2017. G. R. Murugesan*[1], E. Hendel[1], P. Gott[1], C. Pender[1], and U. Hofstetter[2], *[1]Biomin America Inc., Overland Park, KS, [2]Biomin Holding GmbH, Getzersdorf, Austria.*

Mycotoxins are fungal metabolites, often found in feed ingredients that are detrimental to livestock health and performance. The overall financial impact of mycotoxins on crop and livestock losses, monitoring, and interventions are estimated to be as high as 5 billion USD a year in the US and Canada. Mycotoxins could result in subtler impacts even at low levels, compared with the obvious clinical signs observed with higher contamination such as feed refusal, gizzard erosion, fatty liver, impaired feathering, leg weakness, etc., which include predisposing the birds to pathogenic challenges such as necrotic enteritis, coccidiosis, salmonellosis, ascites, etc., by compromising intestinal integrity, and reducing vaccine efficacy through immune suppression leading to reduced performance and increased economic losses. Annual surveys to assess the occurrence levels of mycotoxins in US corn crop were conducted from 2013 to 2017. A total of 1,940 fresh corn and corn byproduct samples collected over the 5 year period were analyzed for Aflatoxins (Afla), Ochratoxin (OTA), and those produced by *Fusarium* fungal species such as Type A-Trichothecenes (A-Trich) including T-2 and HT-2 toxins, Type B-Trichothecenes (B-Trich) including Deoxynivalenol (DON) commonly known as vomitoxin, Fumonisins (FUM), and Zearalenone (ZEN). Due to their consistent presence in the fresh corn, Afla, B-Trich, ZEN, and FUM have been reported. Samples tested positive for at least one mycotoxin gradully increased from 71% to 89%, although co-occurrence of more than one mycotoxin in a sample remained consistent at ~48% over the 5-year period. Co-contamination of mycotoxins is an important factor to consider because they act synergistically, thus the combination has a stronger negative impact than each mycotoxin alone. Positive samples for DON and ZEN have increased ($P > 0.05$) from 27% to 77% and 8% to 31%, respectively. However, FUM and Afla positives were consistent over the 5-year period. While an 'up-down' trend was noticed in the average contamination (ppb) of positive samples for B-Trich, ZEN, and FUM, the overall trend shows an increase in the average contamination levels from 2013 to 2017. Afla had a gradually decreasing trend on its average contamination from 51 ppb in 2013 to 11 ppb in 2017. Overall DON with additional potential concerns from FUM and ZEN pose the greatest mycotoxin threats to poultry health and productivity due to their frequent occurrence in the US corn.

Key Words: deoxynivalenol, fumonisins, zearalenone, fusarium, co-contamination

273 Effects of different sources and levels of copper and zinc on performance and carcass yield of broilers. T. Santos*[1], K. Augusto[2], J. Denadai[1], M. M. Sartori[1], J. Batistioli[1], L. Zanetti[1], A. C. Neto[1], E. Muro[1], G. Pasquali[1], R. Giacomini[1], and J. Sartori[1], *[1]São Paulo State University (UNESP), Botucatu, Brazil [2]Trouw Nutrition, Campinas, Brazil.*

The latest sources of copper (Cu) and zinc (Zn) in broiler nutrition are hydroxychloride (IntellibondC; IBC and IntellibondZ; IBZ), which are less reactive and more stable in the diet and in gastrointestinal tract conditions when compared with inorganic sources. Thus, the objective of the present study was to evaluate the effect of different sources (sulfate and hydroxychloride) and different levels of Cu and Zn on performance (1-to 21 and 1-to 42-d-old), carcass and breast yield, and skin resistance at 42-d-old. A total of 1,792 1-d-old male Cobb chicks were distributed in a completely randomized design with a factorial arrangement of 2x3+2: IBC (15 ppm and 150 ppm) × IBZ (80 ppm, 100 ppm and 120 ppm) + Cu sulfate (15 ppm) × Zn sulfate (120 ppm) or Cu sulfate (150 ppm × Zn sulfate (120 ppm) with 8 treatments and 8 replicates of 28 birds each. The diets were formulated with corn and soybean meal, according to the recommendations of each phase: pre-starter (d1-to 7), starter (d8- to 21), growing (d22- to 35) and finishing (d36-to 42). Data were analyzed in a factorial design 2x3+2 with the statistical software Minitab 16, and the means compared by the Tukey's test,($P < 0.05$). At the end of the experiment, 3 birds per replicate were slaughtered for carcass and breast yield assessment, and the left drumstick skin was used to evaluate skin resistance. Chickens fed diets containing 150 ppm of IBC during d1-to-21-old showed higher body weight gain ($P = 0.006$) and FCR ($P = 0.013$) compared with those fed diets containing 15 ppm of IBC. During d1–42, chickens fed diet containing 150 ppm of IBC and 80 ppm of IBZ had better FCR compared with the treatment using 150 ppm of IBC and 120 ppm of IBZ ($P < 0.0001$). Breast yield ($P = 0.010$) were positively influenced by the inclusion of 100 ppm of IBZ, comparing with 80 and 120 ppm levels. Lower Zn (80 ppm) levels caused a significant higher carcass yield ($P = 0.011$) and skin resistance ($P = 0.046$), comparing with high (120 ppm) levels. There was no significant interaction of Cu and Zn sources on overall growth performance and carcass yields. The results from this study indicate that high IBC levels during the first 21 d for the overall period and the combination of high IBC levels to low IBZ levels have better performance results. The carcass and skin yields were influenced positively by the IBZ levels. Acknowledgment: Trouw Nutrition, Fapesp (2017/00338–2), Capes (scholarship)

Key Words: hydroxychloride, weight gain, feed conversion ratio, skin resistance

274 Impact of inorganic and organic zinc sources on broiler live performance and feed cost. K. Perryman*[1], J. Cohen[1], and N. Sriperm[2], *[1]Micronutrients, McDonough, GA, [2]Nutritional Statistics, Buford, GA.*

A 42-d experiment utilizing 6,864 straight run Yield Plus × Ross 708 broilers (52 birds/pen) was conducted to determine the effects of supplemental Zn program on broiler performance and economics. Dietary treatments (trt) were based on a corn-soy basal diet (0 added Zn) that was supplemented with 1 of 11 Zn source combinations. The first 3 trt were designed as controls: 1) 80 ppm Zn from $ZnSO_4$, 2) 80 ppm Zn from Zn hydroxychloride (ZHXY; Micronutrients USA, LLC), 3) 80 ppm Zn from ZHXY/20ppm Zn from organic source A (OrgA). The remaining 8 trt were arranged as a 2 × 4 factorial (2 Zn levels [100 or 60 ppm] × 4 organic source B (OrgB) replacement levels [0, 20, 30, or 50% of added Zn). Regardless of source, increasing Zn from 60 to 100 ppm resulted in a linear ($P < 0.001$) increase in BW and a quadratic ($P < 0.001$) improvement in feed conversion ratio (FCR). Broilers fed ZHXY had lower ($P < 0.001$) FCR and feed costs/bird than birds fed $ZnSO_4$ when both were fed at 80 ppm. Partial replacement of ZHXY with OrgB resulted in a quadratic ($P < 0.001$) response for FCR. Values were lowest when OrgB replaced 20 or 30% of HXY at both 60 and 100 ppm. Although the replacement of ZHXY with OrgB increased dietary costs, feed cost/bird was lowest ($P < 0.05$) for birds fed a combination of ZHXY and OrgB. No differences ($P > 0.05$) were measured between the 2 organic sources when supplemented at the same concentration. In conclusion, these data indicated that growth performance responded positively to diets with 100 ppm of added Zn, replacing $ZnSO_4$ was beneficial to growth, and that feeding an 80/20 ppm and 70/30 ppm ZHXY/organic blend resulted in the best growth and economic performance. A follow-up trial to determine the effect of these Zn programs on carcass characteristics is warranted.

Key Words: zinc, organic, hydroxychloride, broiler, mineral

275 Effect of supplemental zinc and copper on broiler intestinal lesions and performance under a necrotic enteritis model. D. Neves*, M. Rebollo, C. Zhang, A. Fireman, T. Cheng, L. Linares, and T. L. Ward, *Zinpro Corporation, Eden Praire, MN.*

Necrotic enteritis is a prevalent and growing disease in poultry production, especially in antibiotic-free practices. Necrotic enteritis delays growth, decreases feed efficiency, increases mortality and required vaccinations, and leads to an increase in treatment costs. Due to regulatory restrictions of medication and increased anticoccidial drug resistance, eradication is not fully possible. Reinforcement of intestinal integrity and innate immune response with in-feed supplementation of zinc and copper metal-amino acid complexes (Zn/Cu-AA) could be an opportunity to consider for prevention. This experiment evaluated the effects of zinc and copper source and level in broilers undergoing a necrotic enteritis challenge. A total of 3,808 birds were vaccinated with a coccidiosis vaccine at hatch, followed by in-feed administration of *Clostridium perfringens*, at 17 d of age. Birds were randomly assigned to 1 of 8 treatments: 1) no challenge + 80 ppm Zn as $ZnSO_4$ (NoCh-S); 2) challenge + 80 ppm Zn as $ZnSO_4$ (Ch-S); 3) challenge + 20 ppm Zn as $ZnSO_4$ + 60 ppm Zn as Availa@Zn (Av-Zn-Iso); 4) challenge + 80 ppm Zn as $ZnSO_4$ + 60 ppm Zn as Availa@Zn (Av-Zn-Top); 5) challenge + 80 ppm Zn as $ZnSO_4$ + 14 ppm Cu as Availa@Cu (Av-Cu); 6) challenge + 80 ppm Zn as $ZnSO_4$ + 60 ppm Zn as Availa@Zn + 14 ppm Cu as Availa@Cu (Av-ZnCu); 7) challenge + 80 ppm Zn as $ZnSO_4$ + 120 ppm Cu as tribasic copper chloride (TBCC; TBCC); 8) challenge + 80 ppm Zn as $ZnSO_4$ + 60 ppm Zn as Availa@Zn + 120 ppm Cu as TBCC (Av-ZnTBCC). Data obtained was analyzed by 2-way ANOVA, using the GLM procedure of SAS (v. 9.4). Means were separated by the Tukey test when $P \leq 0.05$. Compared with the challenged control at 35 d of age, birds fed Av-Cu and Av-ZnCu had higher body weight gain ($P < 0.0001$), feed intake ($P < 0.0001$), and feed conversion ratio ($P < 0.0001$). Birds fed Av-Cu, Av-ZnCu, and Av-ZnTBCC treatments had significantly lower ($P < 0.0001$) necrotic enteritis related mortality at 28 d of age, compared with the challenged group. At 21 d of age, birds fed Av-ZnCu had a significantly lower ($P < 0.0001$) average intestinal lesion score, compared with birds consuming sulfates. These results suggest

that in-feed supplementation combining zinc amino acid complex and copper amino acid complex could improve the immune response and protect the intestine against necrotic enteritis in broilers.

Key Words: broiler, copper, metal-amino acid complex, necrotic enteritis, zinc

276 Hydroxy-selenomethionine improves feed conversion of heat stressed finisher broilers associated with enhanced Se bioavailability and antioxidant response. V. Jacquier*[1], M. Briens[1], M. Majdeddin[2], J. Pincemail[3], and J. Michiels[2], *[1]Adisseo France S.A.S., Commentry, France, [2]Laboratory for Animal Nutrition and Animal Product Quality, Gent, Belgium, [3]Centre Hospitalier Universitaire de Liège, Liège, Belgium.*

Selenium (Se), under the form of selenocysteine, is an essential component of selenoproteins, among them major antioxidant enzymes such as glutathione peroxidases and thioredoxin reductases. It was shown that dietary hydroxy-selenomethionine could enrich tissues and blood to a greater extent in selenomethionine and total Se pools and differently affected the expression of the selenogenome as compared with sodium selenite and seleno-yeast. Heat stress in broilers is known to induce oxidative stress. Hence, it was hypothesized that dietary supplementation with hydroxy-selenomethionine will be beneficial to heat stressed finisher broilers due to specific regulation of the selenogenome. A total of 720 one-day-old male Ross 308 broilers were allocated to 3 treatments with 12 replicates (20 birds each). Treatments were: no supplemental Se, Na_2O_3Se at 0.3 mg/kg Se, and hydroxy-selenomethionine at 0.3 mg/kg Se, added to corn-soybean meal basal diets and fed for 39d. A chronic cyclic heat stress model (temperature increase to 34°C with 50–60% relative humidity for 6h daily) was applied in the finisher phase (d25–39). One bird per pen was sampled on d26 (acute heat stress) and d39 (chronic heat stress) to determine selenium concentration in serum, glutathione peroxidase and glutathione redox status in erythrocytes or serum, liver and breast muscle. In the finisher period, when the heat stress protocol was implemented, an improvement in feed efficiency was observed (−5 and 6 points as compared with non-supplemented and Na_2O_3Se supplemented birds, respectively) (both $P < 0.05$). Mortality in the finisher period, which increased substantially with cyclic heat stress, was numerically lowest in treatment with hydroxy-selenomethionine; −3.5 and 4.2% as compared with non-supplemented and Na_2O_3Se supplemented birds, respectively. Se supplementation dramatically increased Se levels in serum, and notably levels were higher for the hydroxy-selenomethionine fed birds as compared with supplemented birds. Corroborating with this, GPx activity in erythrocytes, breast muscle and liver increased multi-fold when diets were supplemented with Se. In conclusion, hydroxy-selenomethionine improved performance of heat stressed finisher broilers, but it remains to be established whether differential effects on the expression of other selenoproteins might be involved.

Key Words: heat stress, selenium, glutathione peroxidase, feed conversion

277 Dietary supplementation of ascorbic acid improve the production performance and immunity of broilers infected with *Salmonella* Typhimurium. L. Gan*, H. Fan, W. Zhen, J. Gao, and Y. Guo, *China Agricultural University, Beijing, China.*

Abstract: A trial was conducted to investigate the effects of dietary ascorbic acid (AA) on production performance, antioxidant capacity and immunity of broilers challenged with Salmonella Typhimurium. The experiment was designed with a 3 × 2 factorial arrangement, i.e., 3 dietary treatments (control, 500 and 1000 mg AA/kg diet) and 2 challenge treatment (with or without Salmonella Typhimurium challenge from 8 to 10 d and 17 to 19 d of age). Samples were collected at age of 21 d. The data were analyzed using the General Linear Model procedure in SPSS version 18.0 (SPSS Inc.), and subjected to 2-way ANOVA in a 3 × 2 factorial arrangement to analyze the main effects of dietary treatments and challenge, and their interaction. The results showed that Salmonella Typhimurium challenge decreased ($P < 0.05$) body weight of broilers on 12 d and 21 d of age, lowered the contents of secretory immunoglobulin A (sIgA) in the ileum ($P < 0.05$), and increased mortality rate and liver salmonella counts($P < 0.05$). Dietary supplementation with 500 or 1000 mg AA/kg diet significantly elevated ($P < 0.05$) body weight on 21 d of age while reduced ($P < 0.05$) the feed conversion ratio during starter period (1–12d). Dietary treatment of 500 mg AA/kg diet significantly increased ($P < 0.05$) sIgA levels in ileal of the infected birds. AA also enhanced ($P < 0.05$) the total antioxidant capacity, reduced ($P < 0.05$) the mRNA expression of NF-κB and IL-1β in cecal tonsil of the broilers. Moreover, addition of AA significantly decreased L-gulonolactone oxidase activity in kidney, whereas elevated the AA contents in the spleen of the birds ($P < 0.05$). In conclusion, dietary addition of AA could alleviate the negative influences such as impaired production performance caused by Salmonella Typhimurium infection through enhancing the antioxidant capacity and immunity.

Key Words: ascorbic acid, *Salmonella* Typhimurium, broilers, immunity, antioxidant capacity

278 Evaluation of performance of broilers fed diets containing soybean oil or palm fat, with graded levels of vitamin A. C. Souza*[1], V. Savaris[1], R. Nunes[1], C. Eyng[1], C. Souza[1], G. Sangalli[1], and J. Oxford[2], *[1]Universidade Estadual do Oeste do Paraná, Marechal Candido Rondon, Brazil, [2]University of Georgia, Athens, GA.*

The effects of 2 sources of lipids (soybean oil *vs* palm fat) and 5 levels of supplementation of vitamin A (VIT A) (0; 3,000; 6,000; 12,000 and 24,000 UI kg^{-1}) on the performance of broilers were studied from 1 to 21 d. This study was designed in a completely randomized factorial arrangement consisting of: 2 sources of lipids (soybean oil or palm fat), 5 levels of VIT A, and 2 control treatments (one for each lipid source) 2,622 d old male broilers were used with each treatment consisting of 10 replicates of 23 birds. The birds did not receive VIT A supplementation until d 3, with the exception the control groups. On d 21 all broilers were weighed and the weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) were determined. The data were analyzed with ANOVA and polynomial contrasts through Statistical software SAS, to test the effects of the lipids source, VIT A levels, and respective interactions. No interactions were observed between source of lipids and VIT A levels, for all variables analyzed. WG was influenced by levels of VIT A, showing a quadratic response, ($-0.00000069VITA^2+0.018758$ $4VITA+746.53$, $R^2 = 0.76$). The highest WG was obtained with 15,401 UI kg^{-1} of VIT A. FI showed quadratic comportment based on inclusion of VIT A ($-0.000000804VITA^2+0.025292VITA+1048{,}43$, $R^2 = 0.80$) the largest FI was obtained with the inclusion of VIT A at 15,267 UI kg^{-1}. FI was higher for soybean oil (1,140 g) than palm fat (1,116 g). For FCR, only effects of source of lipids were observed, with the soybean oil presenting lower FCR (1.389 kg/kg) than (1.418 kg/kg) for palm fat. The utilization of soybean oil may be better than palm fat, this may explain why improved FCR were seen in the soybean oil group. VIT A has an important antioxidant effect, however we were not able to show any interaction with either lipid source. The isolated effect of VIT

A was observed in WG and FI, demonstrating the potential beneficial utilization of VIT A. To obtain optimal FI and WG it is recommended to use 15,267 and 15,401 UI kg^{-1}, respectively, of soybean oil as the source of dietary lipid.

Key Words: broiler, interactions, lipids source, supplementation, vitamin A

279 Effects of 1-alpha-hydroxy-vitamin-D_3 on calcium and phosphorous digestibility in grower diets containing different calcium levels for Ross-708 broilers. V. San Martin*[1], E. Oviedo-Rondón[1], H. Cordova-Noboa[1], J. Cifuentes[1], C. Florez[1], F. Tovar[1], J. D. Fernandez[2], M. A. Wisaquillo[1], I. Cárdenas-García[1], G. Quintana-Ospina[1], and L. Peñuela[3], *[1]North Carolina State University, Raleigh, NC, [2]Premex Inc., Medellin, Antioquia, Colombia, [3]Universidad del Tolima, Ibague, Tolima, Colombia.*

Intestinal absorption and metabolism of calcium (Ca) and phosphorus (P) are influenced by several factors including active vitamin D_3. The 1-α-hydroxy-vitamin-D_3 (1α(OH)D_3) is an active analog of vitamin D_3. This molecule improves performance in broilers affecting mineral absorption and metabolism, but dietary Ca level may affect its activity. The objective of this experiment was to evaluate the effects of dietary Ca content and 1α(OH)D_3 supplementation in grower diets on Ca and P ileal digestibility (dig.) in broilers raised up to 35 d. A total of 1,152 Ross 708 male chicks were placed in 48 pens of 24 chicks each. From placement to 16 d of age chickens were fed one starter diet containing 1α(OH)D_3 at 5 µg/kg of feed. At 17 d, 8 different grower diets were offered. These treatments resulted from a factorial arrangement of 4 levels of Ca (0.54; 0.76; 0.98, and 1.20%), and the supplementation or not of 1α(OH) D_3 (0 *vs*. Five µg/kg feed). At this concentration 1α(OH) D_3 was equivalent to 1.600.000 IU of Vit D_3. These treatments were obtained from one basal diet and Ca content was adjusted by including limestone, dicalcium phosphate and sand in the diet depending on the treatment. The available P of all grower diets was 0.40% with phytase providing 0.12% of available P. Celite was used as an inert marker. Six replicate pens per treatment were used. Pooled ileal samples were collected from 3 broilers per pen at 35 d of age, frozen, lyophilized and later analyzed to estimate Ca and P dig. Data were analyzed in a randomized complete block design with dietary Ca level and 1α(OH) D_3 supplementation as main effects and pen lines considered as block random effect. Two-way ANOVA, mean separation by Tukey's and *t*-test, and regression analyses were conducted. It was detected a main effect caused by Ca level ($P < 0.05$) on dry matter and P dig. The worst dry matter dig. was observed at 0.54% Ca level. Dietary Ca had a positive quadratic effect on dry matter dig. ($P < 0.05$) with a calculated optimum at 0.99% Ca. An interaction effect was detected ($P < 0.05$) for Ca dig. The regression analyses for Ca dig. revealed a negative quadratic effect of Ca levels ($P < 0.01$) in diets without 1α(OH)D_3 inclusion and no changes ($P < 0.05$) in Ca dig. as Ca increased in diets with 1α(OH)D_3. For phosphorous dig., Ca levels had a negative linear effect and 1α(OH) D_3 supplementation increased P dig. as Ca levels increased compared with diets free of 1α(OH)D_3. The highest P dig. was observed at 0.54% Ca. In conclusion, dietary Ca level in grower diets affected calcium and phosphorus dig. The supplementation of 1α(OH)D_3 improved digestibility of phosphorus as Ca levels increased from 0.98% to 1.20%.

Key Words: 1-alpha-vitamin D_3, calcium, phosphorus, digestibility, broilers

280 Revision of the simplified balance method to evaluate phosphorus excretion by pullets and laying hens. M. Hamdi*[2], L. Cloutier[1], R. Chiasson[2], and M. P. Létourneau-Montminy[2], *[1]Centre de développement du porc du Québec, Québec, QC, Canada, [2]Département des Sciences Animales, Faculté des sciences de l'agriculture et de l'alimentation, Université Laval, Québec, QC, Canada.*

Since 2010, guidelines have been developed in Quebec to maximize the benefits and minimize the risks associated with the application of livestock manure to agricultural soils. Within these guidelines, phosphorus (P) content of manure has to be assessed and manure application adjusted to crop needs. To simplify farmers work, a simplified balance method has been proposed to quantifie P excretion as the difference between farm P inputs and outputs. The goal of this work is to estimate the amount of P retained by birds during the pre-laying and laying period, which is a key parameter, and to highlight the factors of variation. A total of 12 different farms of pullets were followed during 19 weeks (W), and 13 laying-hens farms were followed from 19 W until the end of the laying period. Pullets and laying hens received diets that contained 5.3 to 8.4 g and 4.7 to 7.1 g total P/kg respectively. Body weight gain and feed intake were followed during all the experiment and 5 birds per farm were slaughtered at 0, 10 and 19 W for pullets and at 19, 31, 43, 55, 67 W of age for laying-hens. The feathers were removed, whole-body composition in bone mineral content, lean and fat was obtained using dual-energy x-ray absorptiometry (Discovery W Hologic Inc., Waltham, MA, USA) for the pool of 5 birds which was the experimental unit. They were then grinded for body chemical composition in Ca, P, lipid and protein. In addition, for each laying farm, we received 36 eggs in the beginning, in the middle and at the end of laying cycle to analyze different physicochemical characteristics. Data were statistically compared using MIXED procedure of Minitab 18. In average, pullets have taken 1.15 kg during the 19 W compared with 300 g for laying hens from 19 to 67 W. Regarding pullet results, there was no effects of farms on body weight (BW, g) and BMC (g). The P retention efficiency based on carcass analysis and diet varied between 17 and 36%. The regression of body P to BW was fitted: Body P (g) = −0.152+0.00664 × BW (g); $R^2 = 0.98$. In sum, based on the derivative of this equation, 6.6 g P /kg of gain is retain by pullets without farm effect. For laying-hens, the P retention efficiency varied between 13 and 20%, on which only 1% of dietary P is retained by the laying hens compared with egg. The relation between body P and BW is really low ($R^2 = 0.16$) and an average of 4g P/kg gain is retained. Regarding eggs, an average of 1.83 g P/kg fresh eggs is retained. Based on the current results a fixed 6.6 and 4.0 g of retained P per kg BWG is proposed for pullets and laying hens respectively.These values can be used in the simplified balance method to assess P manure and this method would be a valuable tool to improve poultry sustainability.

Key Words: phosphorus, pullets, retention

281 Evaluation of phosphorus absorbability of five feed phosphates in male broilers. J. H. Vieyra[1] and S. Zwart*[2], *[1]FeedProfessionals Ltda, Porto Alegre, RS, Brazil, [2]Aliphos, Vlaardingen, the Netherlands.*

The reduction of phosphorus (P) excretion has been a focus in intensive poultry production systems, therefore knowing absorption coefficient to properly formulate modern diets is crucial.This study was designed to determine the pre-cecal absorbability of 5 different feed phosphates: Dihydrate dicalcium phosphate, anhydrous dicalcium phosphate, and 3 monocalcium phosphates (22.7%P). The experiment was conducted at the Research Facility Carus of the Department of Animal Science of Wageningen University, The Netherlands. The experiment included a pre-experimental period (0–14 d)in which the birds received a standard feed, and an experimental period (14–23 d) in which the trial feeds

were fed. Diets were in agreement with the recommendations of the WPSA protocol for determination of pre-cecal P absorbability. Body weight (BW) and feed intake were determined at the start and end of the experimental period. At 23 d of age, all birds were euthanized by an intravenous injection of T61. The small intestine was ligated and removed from the bird. The content of the terminal ileum was collected from all birds in a pen. Based on the analyzed content in the experimental diets and digesta, the pre-cecal P absorbability was calculated on pen basis. Performance, diet absorbability, absorbability of the test products, were statistically analyzed by ANOVA (GenStat) withpen as experimental unit and diet and P-source as explanatory factor. At the end of the experimental period, broilers fed the basal diet (treatment 1) had a much lower BW and body weight gain (BWG) Also showing the lowest feed intake (FI) and highest feed conversion ratio (FCR). The P source significantly affected the pre-cecal P absorbability of the diets. The pre-cecal absorbability for DCP dihydrate was 81.5%, whereas the P-absorbability for DCP anhydrous was significantly lower, 65.4%. Pre cecal absorbability's for all 3 MCP were similar (78.2–81.3%) and no statistically different from DCP dihydrate (Aliphos Dical+, ALIPHOS DUNKIRK, France). From this study it can be concluded that (1) the basal diet, deficient in P and Ca content, reduced performance, but ideal to evaluate P sources absorbability. The pre-cecal P absorbability of DCP anhydrous (standard DCP) (65.4%) was significantly lower than MCP (80%). DCP dihydrate had the same P ileal digestibility as MCP.

Key Words: phosphorus, apparent ileal digestibility, DCP dihydrate, DCP anhydrous, MCP

282 Vitamin D source effects on broiler live performance, carcass and cut-up yields, and meat quality in Ross 708 male broilers raised up to 42d. V. San Martin, E. Oviedo-Rondón, H. Cordova-Noboa*, F. Tovar, J. Cifuentes, and C. Florez, *North Carolina State University, Raleigh, NC.*

Vitamin D source and metabolism play an important role in modern fast-growing chickens, as it could affect bone development and health leading to depression of live performance. Several Vit D analogs are commercially available, however variable responses on live performance may be observed when used on top of current broiler diets that contain cholecaciferol. One experiment was conducted to evaluate 3 different Vit D analogs and their impact on live performance, meat yield and quality in Ross 708 male broilers raised up to 42d. Four experimental dietary treatments resulted from a common basal diet (control diet) that contained ~4,000 IU D_3 and supplemented with 3 Vit D analogs: 1-α(OH)D_3, 1,25(OH)$_2D_3$, and 25(OH)D_3 at doses recommended by providers. A total of 1,056 one-d-old male Ross 708 broiler chicks were randomly placed in 48 floor pens in groups of 22 chicks/pen. At 7, 20, and 41d, BW and feed intake were obtained and BW gain and FCR were calculated. Flock uniformity was assessed at 41d as the CV% of BW. Additionally, 3 broilers/pen were selected for processing. Carcass and cut-up yields were obtained and whole *Pectoralis major* muscle was used to determine drip and cook loss, pH (6 and 24 h post-slaughter), color (L*, a*, and b*), and myopathies (wooden breast and white striping). Data were analyzed using one-way ANOVA in a randomized complete block design. Mean separation was performed using Tukey's test. Results obtained at 20 d indicated that broilers fed 1,25(OH)$_2D_3$ gain less weight ($P = 0.058$) as compared with broilers fed 1-α (OH)D_3-supplemented diets (977 vs. 998 g). At 41d, broilers fed the control diet were heavier ($P < 0.05$) compared with chickens fed diets supplemented with 1,25(OH)$_2D_3$ and 25(OH)D_3. In addition, FCR was worsened ($P < 0.01$) by 1,25(OH)$_2D_3$ inclusion at this age. No differences ($P > 0.05$) were detected among treatments on feed intake, total mortality, and flock uniformity at 41d. Overall, no differences ($P > 0.05$) were observed in carcass, and cut up yields, and meat quality parameters except for cook loss. The *P. major* samples from broilers fed diets with 25(OH)D_3 lose ($P < 0.05$) more weight at cooking compared with samples from chickens fed the control diet and 1-α(OH)D_3-supplemented feed. Interestingly, 1-α(OH)D_3 inclusion reduced overall mean score for wooden breast myopathy ($P = 0.055$) compared with samples from broilers fed diets with 25(OH)D_3. In conclusion, 1,25(OH)$_2D_3$ supplementation resulted in the worst live performance whereas 1-α(OH)D_3 and 25(OH)D_3 inclusion showed intermediate responses up to 41d. In addition, these Vit D analogs did not affect carcass and cut up yields, and meat quality parameters except for cook loss and wooden breast.

Key Words: 1-alpha(OH)D_3, 25(OH)D_3, 1,25(OH)$_2D_3$, live performance, meat quality

283 Egg qualities of laying hens fed diets supplemented with varying levels of copper (II) oxide. S. Oyefeso*, *Federal University of Technology, Akure, Ondo, Nigeria.*

This study aimed to determine the egg qualities of layers fed diets supplemented with varying levels of Copper (II) oxide (CuO). One hundred sixty Harco black pullets (15 weeks of age) were purchased from a reputable farm and acclimatized for 2 weeks in battery cages and afterward divided into 4 treatments of dietary CuO supplementation (0, 100, 200 and 300 mg CuO per kg diet) for 8 weeks. Each treatment consisted of 40 birds which were further sub-divided into 4 replicates of 10 birds each. Afterward, 3 eggs per replicate were collected for internal and external egg qualities analysis. Data obtained were subjected to ANOVA in a Completely Randomized Design. Inclusion of dietary CuO had no significant ($P > 0.05$) effect on all egg qualities measured except the shell thickness and albumen height. Comparable means (0.29 mm, respectively) for shell thickness recorded at 0 and 100 mg CuO supplementation were significantly ($P < 0.05$) higher than 0.26 mm recorded at 300 mg CuO supplementation. In addition, statistically similar means for albumen height (0.71, 0.68 and 0.72 mm) were observed at 100, 200 and 300 mg CuO, respectively which were significantly lower than 1.20 mm recorded in the control. Hence, the inclusion of CuO in layers diets led to the reduction of egg shell thickness and albumen height.

Key Words: copper (II) oxide, layer, egg quality

284 Effect of 1-α-hydroxycholecalciferol on leg health parameters and bone development in grower diets with different dietary calcium levels for Ross-708. V. San Martin[1], G. Quintana-Ospina*[1], E. Oviedo-Rondón[1], H. Cordova-Noboa[1], J. Cifuentes[1], C. Florez[1], F. Tovar[1], J. D. Fernandez[2], I. Cárdenas-García[1], M. A. Wisaquillo[1], and L. Peñuela[3], *[1]North Carolina State University, Raleigh, NC, [2]Premex Inc., Medellin, Antioquia, Colombia, [3]Universidad del Tolima, Ibague, Tolima, Colombia.*

Calcium (Ca) and vitamin D_3 are nutrients involved in several physiological processes closely related to bone formation and the incidence on bone disorders. The objective of this study was to assess the 1-α-hydroxycholecalciferol (1α(OH)D_3) supplementation effects on grower diets with different Ca levels on leg health parameters (varus, valgus, hock burns and footpad dermatitis, FPD), tibial dyschondroplasia, leg bone relative asymmetry (RA), tibia breaking strength, and tibia ash content in broilers. A total of 1,152 one-day-old Ross-708 male chicks were distributed in 48 floor pens set in 3 rows (16 pens each) and located in a dark curtain-sided house with maximum light intensity of 15 lx. The experimental design consisted in a 4x2 factorial arrange-

ment with dietary Ca levels (0.54; 0.76; 0.98; and 1.20%) and 5µg/kg of feed supplementation (1,600 IU/kg) or not with 1α(OH)D_3 in grower diets as main effects, resulting in 8 treatments with 6 replicates of 24 chicks each. All chickens were fed a starter diet containing 1α(OH)D_3. At 34 d leg health parameters were assessed. At 35 d, 3 chickens per pen were selected for tibia and femur sample collection for deboning and evaluation. Data were analyzed in a completely randomized block design using a 2-way ANOVA analysis. Mean separation was conducted using Tukey's and student's *t*-test. Ca levels affected ($P < 0.05$) valgus incidence. Diets containing 1.2% Ca level resulted in the lowest valgus incidence. An interaction effect ($P < 0.05$) was detected on FPD. Broilers fed diets supplemented with 1α(OH)D_3 containing 0.54% dietary Ca had the lowest FPD score. An interaction effect was also observed on hock burns. Broilers fed low dietary Ca 0.54% without supplementation had greater number of chickens affected with hock burns. No significant effects ($P > 0.05$) on tibial dyschondroplasia were observed. In general, the weight and length RA parameters were not affected ($P > 0.05$) by Ca levels or supplementation, except for RA of tibia weight. The supplementation with 1α(OH)D_3 caused less ($P < 0.05$) asymmetry of bone weight compared with chickens non-supplemented (0.16 *vs*. 0.19). Finally, chickens fed high dietary Ca (1.20%) resulted in a greater tibia thickness ($P < 0.05$), and ash content ($P < 0.05$) as compared with chickens fed diets with low dietary Ca (0.54%), likewise, tibia from those birds were able ($P < 0.01$) to support a higher force stress to be broken. In conclusion, a diet supplemented with 1α(OH)D_3 combined with a low Ca level reduced FPD incidence in broilers and provided per se a more symmetric bone development. However, inclusion about 1.2% Ca level on diets could develop bones with less angulation deformities, greater bone strength and ash content.

Key Words: 1-alpha-vitamin D_3, calcium, leg health, bones, footpad dermatitis

285 Effect of dietary *Pleurotus ostreatus* on growth performance, meat composition and lipid oxidation in meat of broiler chicken. M. Sarker*, S. Sultana, and N. R. Sarker, *Bangladesh Livestock Research Institute, Dhaka, Dhaka, Bangladesh.*

Two hundred forty 1-d-old Cobb broiler chicks were studied with the objectives to know the effect of oyster mushroom, *Pleurotus ostreatus* as dietary feed additives in broiler chickens growth, meat traits and lipid oxidation. The treatments were negative control (basal diet), positive control (basal diet + 25ppm antibiotic, Oxytetracycline), 1.0%, 1.5%, and 2.0% *Pleurotus ostreatus* mushroom powder with basal diet. The broilers were distributed following completely randomized design in floor pens with 4 replications having 12 chicks in each for 3 weeks and provided broiler starter diet with *ad libitum* water supply. Then they were provided finisher diet, rice husk was used as litter materials for the next 2 weeks. The data were analyzed using the computerized SAS version 9.4. Oyster mushroom powder (1%) significantly ($P < 0.05$) enhanced growth performance of broiler chicks compared with other mushroom levels. Among the 3 levels of mushroom powder in the diet it was found that increasing the level of mushroom significantly decreased ($P < 0.05$) the body weight (1824, 1749, 1665 g/b) and feed conversion ratio, FCR (1.96, 2.04, 2.14). Moreover, 1% mushroom powder showed a potential role in contrast of antibiotic on weight gain, FCR and dressing yield (72.88%). Meat composition among the treatments were not affected but significantly reduced the lipid oxidation value (7.63 µmol/100g), compared with other treatments which might be due to the antioxidant potentiality of mushroom. Additionally, less cooking loss in broiler meat and abdominal fat in mushroom treated birds (less than 1.50%) were recorded compared with antibiotic and control diet fed birds. It may be conclude that 1% oyster mushroom powder can be replaced to antibiotic in terms of growth performance, meat yield traits and lipid oxidation value.

Key Words: oyster mushroom, growth performance, antibiotic, lipid oxidation, broiler chicks

286 The supplemental influence of *Saccharomyces cerevisiae* (dried yeast) on the performance of grower pullets. P. Ayomitunde*[3], O. Olorunfemi[1], and H. Gboyega[2], *[1]Federal University of Technology, Akure, Ondo, Nigeria, [2]Federal College of Agriculture, Akure, Ondo, Nigeria, [3]Federal Science/Technical College, Ikare Akoko, Ondo, Nigeria.*

The aim of the study is to determine the performance of grower pullets fed with diets supplemented with various levels of dried yeast. The performance index of grower pullets fed diets containing increasing levels of dried yeast was investigated for 8 weeks. One hundred 9 weeks old growers were studied to determine the growth response and nutrient digestibility as influenced by different inclusion levels of dried yeast in growers rations. The birds were divided into 5 groups and each group further sub-divided into 4 replicates of 5 birds each. The dietary treatments groups consisted of 5 diets. The control diet (T_1) had 0.00% dried yeast supplementation, while T_2, T_3, T_4 and T_5 received 0.25, 0.50, 0.75 and 1.00% respectively. Data obtained were subject to ANOVA in a Completely Randomized Design. Results revealed that birds fed on ration containing 1.00% dried yeast inclusion had the highest ($P <$ 0.05) dry matter intake, final live weight and live weight gain. This was significantly different from other treatments. Hence the inclusion of dried yeast in the diet of the pullets led to added weight and better development of the pullets.

Key Words: supplemental, grower pullet, *Saccharomyces cerevisiae*

287 Effects of the addition of activated carbon in the diets of broiler chickens on the growth performance, nutrient use and the recovery of nitrogen from the manure used as fertilizer. S. Gomez-Rosales* and M. De Lourdes Angeles, *INIFAP, Queretaro, Mexico.*

The objective was to evaluate the production parameters, nutrient balance and the nitrogen recovery in soil and corn forage fertilized with manure of broiler chickens fed increasing amounts of activated carbon (AcCa). The study was divided in 3 phases. In phase 1, 72 male Ross 308 broiler chickens from 25 to 45 d of age were allocated in individual crates and assigned to 4 dietary levels of AcCa (0, 0.15, 0.30 and 0.45%). During the first 15 d of the trial, feed was offered ad libitum; the last 5 d, feed intake was restricted and the excreta was totally collected. The dry matter, nitrogen and energy balance were determined. In phase 2, manure samples from broilers fed increasing levels of AcCa were used as amendment in samples of agricultural soils to determine the nitrogen recovery during a period of 60 d. Two hundred g of dried manure were mixed with 2 kg of agricultural soil in plastic trays under greenhouse conditions. At d 0, 15, 30 and 45, water was added to the mixture to favor the formation of ammonia. At 0 and 60 d, samples of the mixture were taken to determine the dry matter and nitrogen balance. In phase 3, amended soils from phase 2 were used to determine the nitrogen extraction in corn forage. In phase 1, there were 18 replication per treatments and in phase 2 and 3 there were 6 replications per treatment. Results were subjected to ANOVA and linear regression analysis. In phase 1, the weight gain and feed intake of broilers was similar regardless the level of AcCa. The increasing levels of AcCA had a quadratic effect ($P < 0.05$) on the feed conversion ratio ($y = 2.2831 - 1.4848x + 3.0309x^2$; $R^2 = 0.96$). The addition of AcCA had no effect on the dry matter and energy balance, neither on the nitrogen intake and excretion and on the AMEn; the increasing dietary addition of AcCa caused a trend ($P < 0.10$) of a quadratic effect on the nitrogen retention ($y = 59.373 + 14.013x - 21.367x^2$; $R^2 = 0.83$). In phase 2, no differences were observed on the dry matter and nitrogen balance in agricultural soils amended with manure from broilers fed increasing amounts of AcCa. In phase 3, the use of manure as fertilizer from broilers fed increasing dietary levels of AcCa had a cubic effect ($P < 0.05$) on the percentage nitrogen extracted in corn forage ($y = 0.7305 + 6.7468x - 40.111x^2 + 54.966x^3$; $R^2 = 0.99$). In summary, the feed efficiency and nitrogen retention in broilers were greater at AcCa levels of 0.24 and 0.33% and the extraction of nitrogen in corn forage fertilized with manure was greater with manure from broilers fed 0.15% AcCa.

Key Words: broiler, activated carbon, nitrogen retention, manure, nitrogen recovery

289 Yeast cell wall immunomodulatory and intestinal integrity effects on broilers challenged with *Salmonella* Enteritidis. B. Beirão[2], M. Ingberman[2], M. Bonato*[1], L. Borges[1], and R. Barbalho[1], *[1]ICC Brazil, São Paulo, São Paulo, Brazil, [2]Imunova Análises Biológicas Ltda ME, Curitiba, Paraná, Brazil.*

The objective of this study was to evaluate the immune effects and the dynamics of intestinal integrity in broilers challenged with *Salmonella* Enteritidis (SE) and treated with yeast cell wall (YCW). One hundred birds were housed in isolators at 1 d of age and divided into 4 treatments: G1- Birds challenged with SE; G2- Birds not challenged and supplemented with YCW (*Saccharomyces cerevisiae*, IMW50 from ICC Brazil, at 0.5 kg/MT); G3- Birds not challenged and not treated/medicated and; G4- Birds challenged with SE and supplemented with YCW (same inclusion). The challenge was administered orally at 2 d with 10^8 cfu per bird. SE was quantified in crop and ceca contents at 8, 14 and 21 d. Circulating lymphocyte and monocyte subsets, as well as phagocytic cells, were evaluated at the same time ages. Samples of ileum, ceca, and liver were collected at 14 d (8 birds/treatment) for histopathology. Specific IgA for SE in feces was evaluated also at 14 d. Intestinal mucosa permeability was assessed in 8 birds/group at 4, 8, 14 and 21 d by the passage of a marker (Dextran-FITC, 3–5 kD) from the intestinal lumen to blood. The data were analyzed by ANOVA and the means compared by Tukey's test at 5% of significance. At 4 d, G1 presented the highest intestinal permeability (significantly different from the treated group [G4]). Circulating leukocytes counts were higher in the non-SE challenged groups (G2 and G3). Despite this, challenged groups consistently presented higher numbers of various cell subtypes, especially at 14 d (APCs, monocytes, suppressor monocytes, and the series of helper T lymphocytes and cytotoxic T lymphocytes). Treatment was effective in controlling leukopenia and in preventing some of the immune subset fluctuations provoked by the challenge, such as for APCs and cytotoxic cells. The number of phagocytic cells was increased by the challenge at 8 d, while the YCW decreased this effect. G4 presented the highest number of reactive animals, as well as the highest level of anti-salmonella IgA. The challenge induced marked inflammatory responses in the intestine and liver (assessed by lymphocyte counts, section area, goblet cell counts, tissue architecture). Treatment was effective in improving tissue inflammatory signs such as lymphocyte infiltration in the cecum, but not liver. In summary, the challenge with SE was effective in inducing changes in all evaluated systems; however, intestinal integrity and some immune parameters (APCs, suppressor monocytes, IgA) were improved by dietary YCW in challenged birds.

Key Words: *Saccharomyces cerevisiae*, poultry, intestinal permeability, IgA

290 Dietary inclusion level effects of a phytogenic premix on meat and intestinal biomarkers of broiler antioxidant capacity. K. Mountzouris*, V. Paraskeuas, E. Griela, G. Papadomichelakis, and K. Fegeros, *Agricultural University of Athens, Athens, Greece.*

The aim of this study was to assess the effects of dietary inclusion level of a phytogenic premix (AncoFit – Poultry, ANCO Animal Nutrition Competence GmbH, Austria) on broiler liver and meat lipid oxidation and total antioxidant capacity (TAC). In addition, intestinal mucosa TAC and gene expression of antioxidant enzymes (i.e., CAT, SOD, GPX2, GPX7) and transcription factor Nrf2 were profiled along the broiler small intestine and ceca. Depending on phytogenic premix (PP) inclusion level (i.e., 0, 750, 1000 and 2000 mg/kg diet) in a 3 stage feeding program formulated to meet Cobb 500 nutritional requirements, treatments were: PP-0, PP-750, PP-1000 and PP-2000. Feed and water were available *ad libitum*. Each one of the 4 treatments had 125 broilers arranged in 5 replicates of 25 chickens each. At 42d, 4 birds per treatment replicate were pooled for biochemical analyses (i.e., via TBARS and Oxygen Radical Absorbance Capacity analytical methods), while 2 birds per treatment replicate were analyzed for gene expression (i.e., via intestinal mucosa RNA isolation, reverse transcription to cDNA and quantitative Real Time PCR). Data were analyzed by ANOVA, taking the treatment as fixed effect. Statistical significant effects ($P \leq 0.05$) were further analyzed and means were compared using Tukey HSD test. In addition, polynomial contrasts tested the linear and quadratic effect of PP inclusion levels. Lipid oxidation was delayed in a linear pattern with increasing PP inclusion level in breast ($P_L = 0.020$) and liver ($P_L = 0.046$). Moreover, the PP inclusion level resulted in higher breast ($P = 0.005$), thigh ($P = 0.002$) and liver ($P = 0.040$) TAC. In particular, breast and thigh TAC increased in a quadratic pattern reaching plateau at PP-1000, whereas liver TAC continued to increase linearly. Intestinal mucosa TAC was higher in duodenum ($P = 0.011$) and ceca ($P = 0.050$) in PP-1000 compared with PP-0. Gene expression of SOD was upregulated in the duodenum ($P = 0.027$), jejunum ($P = 0.026$) and ceca ($P = 0.023$) in PP-1000 and PP-750 compared with PP-0. Expression of GPX2 was upregulated in the duodenum ($P = 0.032$) and jejunum ($P = 0.013$) in PP-1000 and in ceca ($P = 0.006$) in PP-2000 compared with PP-0, respectively. In addition, Nrf2 was upregulated in ceca ($P = 0.024$) in PP-1000 compared with PP-0. Overall, a consistent PP inclusion effect on meat, liver and intestinal antioxidant capacity has been shown with PP-1000 being the most effective.

Key Words: chicken, phytogenic, antioxidant, carvacrol, thymol

291 A commercial mixture of plant extracts and fatty acids, modulates immune responses, through inhibition of nitric oxide production, and induction of host defense peptide synthesis in an in vitro and in vivo model, respectively. A. Khadem*[1], J. Al-Saifi[2], B. Letor[2], S. Bauwens[2], M. Sevastiyanova[2], F. Combes[1], G. Zhang[3], and N. N. Sanders[1], *[1]Ghent University, Merelbeke, Belgium, [2]Innovad NV/SA, Berchem, Belgium, [3]Oklahoma State University, Stillwater, OK.*

Earlier, it was proposed that effective growth promoters are modulators of immune responses, thus potentially saving energy for growth. This study aimed to evaluate the anti-inflammatory and immunomodulatory effects of a commercial mixture of plant extracts and fatty acids (Lumance) in an in vitro and in vivo experiments. The monocytic murine cell line RAW 264.7 was treated in vitro with medium containing LPS to stimulate inflammation in the absence or presence of Lumance and assayed for nitric oxide (NO) production. The in vivo experimental protocol was approved by the local ethics committee for animal experiments at the Oklahoma State University. One hundred and 80 1-d-old male Cobb chicks were purchased from a commercial hatchery and grown over a 10-d experimental period. The experiment was performed in 20 pens with 9 broilers per pen in an environmentally controlled room. Broilers were fed a non-medicated corn-soybean basal mash diet supplemented with or without Lumance. On d 2 and 4, birds from each treatment were sacrificed with 2 birds/pen. A segment of the crop and jejunum was taken and snap frozen in liquid nitrogen for RNA extraction and subsequent real-time PCR analysis of the expression levels of the host defense peptide (HDP) or Avian β-defensin 9 (AvBD9) gene. Statistical differences between treatments were determined by *t*-test using SPSS software. In vitro, Lumance inhibited LPS-induced NO production with an IC50 value of 1471 (95% CI 716, 2226) mg/L. The inhibition of NO production was not the consequence of cell death because it was established earlier using the tetrazolium salt method that cell viability was not affected by Lumance. The overall consequence of HDPs function is the control of inflammation along with maintenance of the immune responses required for resolution of infections. In vivo, real-time PCR results showed that, relative to control on d 2, Lumance increased AvBD9 gene expression in the crop by 65-fold, but the induction was almost reduced to the basal level on d 4. Higher expression

of AvBD9 gene in crop is likely due to the synergistic effect between short- and medium-chain fatty acids and other plant extracts. However, it is unknown why the ability of Lumance to induce AvBD9 get subsided in the crop by d 4. Although the HDP induction is expected to reduce in the lower GI tract as fatty acids gradually get absorbed and metabolized, it is surprising to see no obvious effect on AvBD9 expression in the jejunum on either d 2 or 4. In conclusion, Lumance showed a strong capacity to reduce inflammation in in vitro test and induces HDP expression in crop in vivo. Therefore, Lumance may have beneficial effects on gut health and immunity.

Key Words: host immune response, anti-inflammatory effect, immunomodulatory, nitric oxide, host defence peptide

292 Phytogenic feed ingredients improve birds' resilience to coccidiosis infections. J. Dirk van der Klis*[1], L. Jungbauer[1], S. Appleton[2], and A. Mueller[1], *[1]Delacon Biotechnik GmbH, Steyregg, Austria, [2]Delacon Biotechnik GmbH, Carlisle, PA.*

Resilience of birds against intestinal infections is key to maintain production performance under challenge conditions. Phytogenics comprise a large group of natural bio-active compounds that stimulate the intestinal tract as first line of defense at different levels: By reducing the pathogenicity of the intestinal microbiome, by improving the integrity of the enterocytes and by stimulating the gut associated immunocompetence. The effect of a blend of quillaja saponins, and selected essential oils from the myrtaceae plant family were tested in male Cobb 500 broilers, using a combined *Eimeria* and *Clostridium perfringens* (Cpf) infection in a 42-d floor pen study (7 replicate pens per treatment; 50 birds per pen) at Southern Poultry Research, Inc., Athens, USA. This combined infection was done to test the resilience against primary and secondary intestinal infections after a coccidiosis challenge. All birds were spray-vaccinated with Coccivac B, in part followed by a Cpf challenge at d 19, 20 and 21. Production performance was measured during the 42-d trial period. Necrotic enteritis lesions were scored in 3 birds per cage on d 21. Oocysts excretion was measured by counting oocysts per g feaces (OPG) on the same day. Treatments were 'not Cpf infected and not treated' (NINT), 'Cpf infected not treated' (INT), 'Cpf infected and treated with 50 g/t BMD' (IT) and 'Cpf infected and treated with the phytogenic blend' (IPB). Birds were fed a 3-phase corn/soy crumbed (starter) or pelleted (grower/finisher) diet for ad libitum intake. Body weight gain (BWG) was reduced from 2219 to 2056 g ($P < 0.05$) and feed conversion ratio (FCR) increased from 1.789 to 1.866 ($P < 0.05$) due to the Cpf infection. Dietary addition of BMD or the blend of phytogenic actives resulted in similar production performance as NINT, IPB having a numerically lower FCR (1.754) than NINT (1.789) or IT (1.781). IPB results revealed a significant reduction in OPG (46440 versus 80500 oocysts/g in the INT group), presumably due to less favorable intestinal conditions for Eimeria reproduction. Results of this study indicate the resilience of birds against intestinal pathogens was improved by feeding this phytogenic blend.

Key Words: necrotic enteritis, broiler, production performance, phytogenics, oocysts excretion

293 Effects of a commercial xylanase-direct-fed-microbial feed additive on gut health and livability of broilers raised under severe coccidiosis challenge conditions. B. Nusairat*[1], M. Schwartz[2], J. Tyus[1], and J.-J. Wang[1], *[1]BioResource International Inc., Durham, NC, [2]Schwartz Consulting Services Inc., Louisburg, NC.*

A study was conducted to evaluate the effects of a proprietary blend of xylanase and multi-strain *Bacillus* direct-fed microbial (DFM), (Enza-Pro, EP, BioResource International Inc.) supplemented to corn-soy-based broiler diets on the live performance and severity of intestinal lesions of broilers raised in battery cages to 28 d. A total of 512 d-old male Ross 708 broiler chicks were assigned to one of 5 dietary treatments, with 7 replicate cages per treatment except treatment 1 (unchallenged positive control which contained 4 replicate cages) and 16 birds per cage. Cage size is 140 cm × 280 cm. Diets were formulated in 2 phases (Starter d 0–14, and Grower d 15–28). Dietary treatments were unchallenged positive control (PC), challenged negative control (NC), NC + 100 g/MT EP, and NC + commercial coccidiostat 42 g/MT (CS). Birds fed NC diets were orally gavaged and challenged at 1 and 7 d of age with a live vaccine containing multiple strains of *Eimeria* spp. Data were analyzed as a randomized complete block design. At 28 d, mortality was reduced ($P < 0.05$) in birds fed NC + EP diet compared with birds fed the NC diet (10.3 vs 1.3%). Supplementation of EP reduced ($P < 0.01$) upper-tract intestinal lesions by 36% compared with NC at 14 d. The overall trend was maintained for mid-tract intestinal lesions to 21 d for birds fed NC diets supplemented with EP. The 28-d FCR among birds of EP treatment was improved by 7 points compared with NC. Results of the current trial suggest that this blend of xylanase and probiotics improves broiler performance by significantly reducing mortality and improving gut health under severe challenge.

Key Words: broiler, xylanase, direct-fed microbials, lesions, mortality

294 Anticoccidial efficacy of a multi-strains yeast fractions product fed to commercial broiler chickens exposed to a mixed challenge of *Eimeria acervulina*, *E. maxima,* and *E. tenella.* G. Mathis*[1], B. Lumpkins[1], V. Demey[2], and E. Chevaux[2], *[1]Southern Poultry Research Inc., Athens, GA, [2]Lallemand, Blagnac, France.*

Coccidiosis is a major enteric disease that poultry industry tries to control as much as possible, since it is also a risk factor for necrotic enteritis. Anticoccidial drugs remains the most affordable solution but the development of some coccidia resistance encouraged the application of alternate strategies. Besides management programs including vaccination, nutritional solutions have been evaluated. The potential of a proprietary multi-strains yeast fractions product (YANG) is assessed in the present study using a randomized block design with 9 replications of 10 birds per cage. The treatments were nonmedicated non-infected (NMNI), nonmedicated infected (NMI), YANG dose 1 (400 g/t), YANG dose 2 (1000 g/t), YANG dose 3 (3000 g/t). Day of hatch male chicks (Ross 708) were placed into cages and issued treatment feeds (D0). Treatment feeds were available ad libitum throughout the experiment (D0–28). On D14, birds were challenged with *E acervulina* (EA), *E. maxima* (EM) and *E. tenella* (ET) at an average total of 150,000 oocysts, while NMNI birds received 1 mL of distilled water. On D5 and D6 post-infection (PI), fecal OPG was counted. Lesion scoring and Bursa of Fabricius (BF) weight were reported for D6 PI. Feed conversion PI (D14–20 and D14–28) was reduced ($P < 0.05$) for YANG, doses 2 and 3 being the most consistent over time against NMI. Lesion scores for all 3 doses were reduced versus NMI. Specifically, ET was reduced for YANG dose 2, EA for YANG dose 3 and EM by all doses. A negative correlation was reported for OPG with YANG dose, EM being more sensitive to YANG doses 2 and 3. BF (% body weight) was higher for YANG dose 2 when compared with NMI. Coccidiosis related mortality was numerically but non-significantly reduced by all YANG doses. Specific nutritional solution such as YANG helps birds to mitigate coc-

cidiosis negative effect, but the animal response looks dose sensitive, with 1000g/tonne as the most consistent.

Key Words: yeast fractions, coccidiosis, *Eimeria*

295 Effect of different feed additives on performance and health status of broilers challenged with *Eimeria tenella*. B. Canal*[1], L. Mesas[1], M. Gracia[2], M. Puyalto[1], and J. J. Mallo[1], *[1]Norel, Madrid, Spain, [2]IMASDE, Madrid, Spain.*

With antibiotic growth promoters (AGPs) being progressively banned, interest in natural alternatives like essential oils (EO) is exponentially increasing due to their many beneficial properties as feed additives. The aim of this study was to evaluate the effects of a combination of ginger, oregano, garlic and clove essential oils (EO-FIT Poultry, NOREL), and saponins as feed additives, defining their impact on performance and health status of broilers challenged with *Eimeria tenella*. One-year-old broiler chickens were assigned to 4 treatments with 3 replicates of 15 birds each during 42 d. These treatments were: T1 – Negative control [NC]: basal diet, T2 – Positive control [PC]: NC + challenge, T3 – PC + essential oils (EO-FIT Poultry; 1kg/ton), T4 – PC + saponins; 1 kg/ton. On 14d birds from treatments 2 to 4 were challenged with 8,500 oocysts of *Eimeria tenella*. Performance was measured at 21d, 28d and 42d of age. Intestinal lesion scoring was carried out on 21d by necropsying 15 birds per treatment. At 28d, 15 additional animals per treatment were euthanised and oocysts per gram (opg) of cecal content quantified. Results were analyzed by one-way ANOVA using GLM procedure of SSPS v. 19.0. Weights obtained from 0 to 42d were not significantly different, although there was a trend (P = 0,0817) suggesting an improvement in weight between animals from EO treatment (3,037^{x}g) and those treated with saponins (2,804^{y}g), with no significant differences compared with NC (2,976^{xy}g) or PC (2,982^{xy}g). Globally (0–42d), performance figures were similar for all those challenged animals regardless of the treatment except for the FCR, which was significantly higher (P = 0.0126) for birds treated with saponins (1.61^{c}) compared with the NC and EO treatment (1.46^{a} and 1.53^{ab} respectively, 1.55^{bc} for PC). No significant differences in the Johnson & Reid lesion scoring were observed between challenged treatments, although the average gut lesion from the PC and saponin treatment (2.07 for both) was 23.95% higher than lesions from birds treated with EO (1.67). No lesions were registered for NC treatment. Birds from the NC and EO treatments had significantly lower oocyst presence in cecal content at 28d compared with the PC (1,286^{b}, 10,753^{a}, 546^{b} and 2,808^{ab} opg for NC, PC, EO and saponin, respectively; P = 0.0084). These results show that essential oils were able to affect the parasite's life cycle, although they could not significantly improve production performance or lesion scoring of challenged birds. Further investigation is required to gain more knowledge about how *Eimeria tenella* is affected by essential oils.

Key Words: essential oils, *Eimeria tenella*, coccidiosis, intestinal health

296 The effect of dietary sodium bisulfate and monensin on growth performance of experimental *Eimeria* inoculated broiler chickens. M. Talghari, S. Rahimi*, M. A. K. Torshizi, and A. Mozafari, *Tarbiat Modares University, Tehran, Iran.*

The coccidiosis is one of the most important parasitic diseases in birds. In addition to direct damage of disease, such as increasing mortality and reducing the quality of carcasses; annually entail huge amounts of money for the poultry industry. In this study we evaluated the effect of sodium bisulfate and monensin on growth performance of broilers experimentally inoculated by *Eimeria*. Two hundred forty 1-d-old chicks (ROSS 308) divided into 4 treatments and 4 replications (15 birds per replicate). Treatments included: 1. Control (fed basal diet without any feed additive), 2. Sodium bisulfate (5 g/kg of basal feed) 3. Monensin (1 g/kg of basal feed), and 4. Sodium bisulfate + monensin (5 g /kg and 1 g /kg of basal diet, respectively). All birds were orally inoculated by *Eimeria* (%70 *tenella*, %15 *maxima*, %15 *necatrix* and *acervulina*- 50,000 oocysts per bird) on 14 d of age. Data were analyzed in a completely randomized deign and significant differences in means were separated by Duncan test (P < 0.05). Orthogonal contrast of combination of monensin and sodium bisulfate versus individual usage of each was carried out to reveal the potential interactions between monensin and sodium bisulfate on performance of challenged broilers. Monensin alone and in combination with sodium bisulfate significantly improved the overall feed conversion ratio compared with control (P < 0.01). The likely synergistic effect of monensin and sodium bisulfate was observed in 1-14d and 14-28d body weight gain, and feed intake and 29-42d feed conversion ratio (P < 0.05). In conclusion, the combination of monensin and sodium bisulfate dietary supplementation is recommended when the potential risk of coccidiosis is expected.

Key Words: broiler chicken, coccidiosis, monensin, sodium bisulfate

297 An evaluation of *Clostridium perfringens* challenged broilers fed diets supplemented with a standardized blend of bioactives, allium and eugenol, or BMD shuttled to Stafac. J. Maurin[1], M. D. Sims[2], J. Welsh[3], and D. M. Hooge*[4], *[1]Pancosma S.A., Geneva, Switzerland, [2]Virginia Diversified Research Corp., Harrisonburg, VA, [3]DVM, Keezletown, VA, [4]Consulting poultry nutritionist, Eagle Mountain.*

A standardized blend of bioactives, allium from garlic and onion tincture plus eugenol from cloves, is useful to improve digestibility and protect against clostridia. A 42-d pen trial was conducted with 1,980 straight-run Cobb 500 chicks using 4 dietary treatments, 10 pens/treatment, and 33 chicks/pen (1.22 × 1.52 m) to evaluate performance. A randomized complete block design ($P \leq 0.05$) was used with Tukey's test (P = 0.05) to separate means. A continuous 24 h daily lighting regimen was used. Live coccidia vaccine was given at placement (d 0), used litter with *Eimeria* and bacterial pathogens as a mild disease challenge was applied to each pen on d 4, and *Clostridium perfringens* (toxin producing strain; dose titrated for 5–10% mortality; in 3 mL nutrient broth/bird twice daily by oral gavage) was given on d 17–19. Economical, low protein pelleted diets (0–14, 15–28, 29–42 d) based on corn, soybean meal, meat and bone, DDGS, and soybean oil were fed. Treatments were: basal diets (Uninfected Control, UC), basal diets with challenge (Infected Control, IC), basal diets + allium and eugenol (XT Blend, 50 g/mT each), or basal diets + BMD 55 g/mT (0–28 d) or Stafac 22 g/mT (28–42 d). Live performance was measured weekly. Necrotic enteritis (NE) lesion scoring, 0 normal to 4 most severe (3 birds/pen per age), was done on d 21 and ~35. Challenge model was effective with poorer BW from 21 d and feed conversion (FCV) and mortality-adjusted FCV (MAFCV) from 14 d. The XT Blend diets improved 42 d BW *vs*. IC (1.835 *vs*. 1.508 kg) with antibiotic diets intermediate (1.714 kg). Antibiotic or XT Blend diets improved 0–42 d FCV and MAFCV *vs*. IC. Antibiotic or XT Blend blend diets reduced 0–42 d NE mortality % *vs*. IC. Antibiotic diets lowered ~35 d NE scores *vs*. IC with XT Blend diets intermediate. Under clostridial challenge, bioactive XT Blend diets improved broiler final BW, FCV, MAFCV, and NE mortality % *vs*. IC.

Key Words: *Clostridium perfringens*, essential oil, eugenol, garlic, phytogenic

298 Effects of NeutraPath, a newly developed formulated feed additive, on the performance of broiler chickens with experimental necrotic enteritis. H. Xue*, S. Ching, E. DeBoer, S. Johnston, and R. Cravens, *Amlan International, Vernon Hills, IL.*

Two studies were performed to evaluate the effects of a formulated feed additive, NeutraPath, compared with a commercial antibiotic, BMD, on the growth performance of broiler chickens challenged with experimental necrotic enteritis (NE). In the 28-d experiments, 224 (Study 1) and 240 (Study 2) 1-d-old male Cobb 500 broilers were randomly blocked and assigned to one of 4 treatments: 1) non-challenged control (CON); 2) challenged control (CH); 3) CH birds fed BMD (55 ppm bacitracin); and 4) CH birds fed NeutraPath, a proprietary blend of ingredients that has antimicrobial, disruptive quorum-sensing and enterosorbent properties. There were 4 blocks and 7 (Study 1) or 8 (Study 2) pens with 8 birds/pen. Each bird received 5,000 Eimeria maxima oocysts on d 13 (Study 1) or d 14 (Study 2). Challenged birds received ~108 cfu of Clostridium perfringens on d 18, 19 and 20 (Study 1) or d 19, 20 and 21 (Study 2). Intestines from 3 birds/pen were examined for NE lesions on d 21. Differences among groups were tested using one-way ANOVA. Compared with CH control, NeutraPath significantly reduced mortality (54% vs. 27% and 26.6% vs. 4.7% for Studies 1 and 2, respectively; $P < 0.05$). Mortality among NeutraPath-treated birds did not differ from BMD-treated in either study, and was not statistically different from CON birds in Study 2. Necrotic enteritis-related lesion score of the NeutraPath group was significantly lower than the CH control (0.6 vs. 1.2, $P < 0.05$) and was not different from the BMD treatment in Study 1. However, in Study 2, no statistical difference in lesion scores was observed among CH control, BMD and NeutraPath groups. During the post-challenge period and the overall 28-d period of both studies, challenged birds fed NeutraPath had greater weight gain compared with the CH control ($P < 0.05$) and did not significantly differ from BMD-treated birds in either study. Feed intake was not significantly different between NeutraPath and BMD groups for either study. In both studies, birds receiving NeutraPath had significantly improved FCR compared with the CH control ($P < 0.05$) but did not differ from birds treated with BMD during either the post-challenge phase or overall. The results of these 2 studies demonstrate that NeutraPath confers therapeutic benefits that are comparable to BMD in improving performance of broiler chickens challenged with experimental necrotic enteritis. As such, NeutraPath can be an effective, natural alternative to in-feed antibiotics.

Key Words: necrotic enteritis, alternative to in-feed antibiotics, mortality, weight gain, feed conversion ratio

299 In vivo collagen synthesis in *Pectoralis major* determined at different ages of grow-out cycle for high yielding broiler. P. Maharjan*, G. Mullenix, M. Schlumbohm, K. Hilton, A. Beitia, J. Weil, M. Cortes, B. Mallamann, J. England, C. Owens-Hanning, and C. Coon, *University of Arkansas, Fayetteville, AR.*

A study was conducted to understand in vivo soluble (S-) and insoluble (I-) collagen fractional synthesis rate (FSR) in breast muscle *(Pectoralis major*) using stable isotope flooding method at different ages (d 21, d 28, d 35, d 42, and d 57) in Cobb 700 broiler grow- out cycle. Birds were fed standard Cobb 700 diet throughout the study except the amino acid levels (120% of recommended level). FSR (% h^{-1}) was determined using precursor–product method: ΔEp /A* 1/t, where ΔEp is enrichment change between baseline tissue sampling and tissue sampling taken at time 't' post infusion of tracer, and A is the area under the curve derived from the decay in plasma enrichment over time 't'. Birds (n = 5) were infused with 1-^{13}C proline as tracer amino acid at each sampling age, and isotopic enrichment ^{13}C-to-^{12}C ratio for muscle and plasma samples were measured using the ratio of fragments 287 to 286 derived from GC MS. Collagen in breast tissue muscle for sampling ages was quantified using hydroxyproline (HP) assay. Breast tissue samples were also subjected for histomorphology. For the last 3 sampling occasions, breast muscle tissue was also subjectively scored for manifestation of woody breast myopathy conditions. Total collagen (DM basis) increased linearly (R^2 = 0.78) with 12. Sixteen (±0.96) µg/mg at d21 to 31.66 (±4.86) µg/mg at d57. Histo-micrographs showed greater accumulation of collagenous tissue in perimysial and endomysial regions of muscle fibers as bird aged. Higher collagen FSR values determined at earlier ages of broilers (d 21 and d28) were higher than later ages (d 35, d 42 or d 57) ($P < 0.01$). FSR (% h^{-1}) at d 21 were 0.43 (±0.033) and 0.22 (±0.017) and at d 28 were 0.25 (±0.019) and 0.33 (±0.014) for S- and I- collagen fractions. FSR values for S- and I- collagen tend to decrease with age. Greater bird-to-bird variation existed in FSR values, at d 35 and d 42 compared with variation in FSR in younger broilers. FSR values at d 57 were not detectable (0% h^{-1}) on hourly basis. FSR values were not observed to be correlated with WB scoring for the analyzed samples (R^2 = 0.01). Overall, the study indicated a lower fractional synthesis rate for I-collagen with age during the grow-out cycle.

Key Words: collagen, synthesis rate, broiler

300 Real-time machine vision system for automatic and high throughput screening of woody broiler breast fillets. S.-C. Yoon*, B. Bowker, K. Lawrence, and H. Zhuang, *USDA-ARS, Athens, GA.*

The current method for woody breast (WB) detection is by tactile evaluation and product handling. The development of a rapid, non-invasive sensing technique to objectively detect WB meat would greatly enhance the industry's ability to manage the problem through product segregation and to provide real-time measures of incidence rates that may provide insight into production and management practices linked to the myopathy. This study is concerned with a real-time machine vision system for automated online WB detection. Through a series of trials, a cost-effective, commercially viable technology has been developed for the automatic and high throughput detection of WB in boneless skinless breast fillets. Samples for trials were collected from the deboning line of a commercial plant and assigned WB scores from 1 to 3 in 0.5 score increments (normal = 1, moderate = 2, severe = 3). The technology includes an innovative machine vision system operating in high speed imaging mode (200 Hz) that allows the capture of moments of physical deformation of fillets. These trials have 1) identified a key physical property (muscle rigidity) that can be used to accurately segregate fillets based on WB, and 2) determined that this physical property can be accurately and consistently measured using a computer vision system. The developed system was designed to measure the degree of bend in breast fillets falling over the edge of a conveyor belt at a speed up to 40 m/min and differentiate between normal and WB fillets continuously moving in a single file formation. The machine vision system consists of a digital camera, a lighting system, a computer, and software (C++). Bending differences between normal and WB fillets were analyzed from images of individual fillets falling over the moving conveyor belt. Shape descriptors characterizing the fillets dynamically bent near the roller axle of the conveyor were developed. An initial trial implied that bending property is closely related to muscle rigidity and WB score. Preliminary results obtained with 326 fillet samples over 3 trials indicated up to 98% overall classification accuracy between normal and severe WB fillet groups, where the accuracy was measured by (number of correct predictions)/(total number of predictions). An equal number of normal, moderate WB and severe WB fillets were tested for each trial. During the trials, 3 line speeds (3, 15 and 30 m/min) were evaluated. A real-time imaging study showed that the system is capable of processing more than 100 fillets per minute at a line speed of 40 m/min with accuracy rates similar to previous trials. The technology is patent pending and its commercialization effort is underway with a poultry equipment manufacturer.

Key Words: woody breast, broiler, detection, machine vision, poultry

301 Sensory quality of vacuum-packed cured-smoked chicken fillets as influenced by *Capsicum annuum* extract over storage. O. Olusola and A. Adeshola*, *University of Ibadan, Ibadan, Oyo, Nigeria.*

Unpredictable power supply typical of developing countries exposes meat and meat products to temperature abuses during storage. There is a need for development of shelf stable meat products to preserve raw meat and ensure continuous supply of protein to the teaming populace. Sensory analysis, as one of the tools for quality control in meat products development allows for effective quality and acceptability assessment over a storage period. This study evaluated the preservative effect of red pepper (*Capsicum annuum*) extract on sensory quality of vacuum-packed cured-smoked chicken fillets from improved indigenous meat-type chickens over a 60-d storage at room temperature. Experimental treatments had batches of chicken fillets cured with brine solution containing 0.015% nitrite (T1-positive control), no nitrite/extract (T2-negative control), 0.15% (T3), 0.30%(T4), 0.45% (T5), 0.60% (T6) and 0.75% (T7) capsicum extract. An assessment of chicken fillets for changes in quality was carried out by a 10-member trained sensory panel using a 3-point descriptive scale on d 0, 15, 30, 45 and 60 of storage. Data were analyzed using 2-way ANOVA ($P < 0.05$). Significant ($P < 0.05$) effect of treatments were observed in color and slime formation on prepared fillet samples while odor, visible microbial growth, muscular elasticity and overall quality were not ($P < 0.05$) affected. Duration of storage, however had ($P < 0.05$) effect on all parameters measured. No slime formation was observed on d 0 for most treatments up to d 30 of storage but was visible on some surfaces for T2 (1.9) on d 15, T4 (1.8) and T1 (1.7) on d 45 and 60 respectively. Chicken fillets' color, odor and muscular elasticity scores were altered over the storage period for all

treatments. Panellists recorded darker brown color for chicken fillets as storage days progressed, pink to light red (3.00) on d 0 to dark brown (1.49) on d 60, on-set of off-odour at d 45 (2.39) and softer fillets with average muscular return at d 30 (2.21). Overall quality reduced from 3.00 on d 0 to a range of 1.3 to 2.1 for all treatments on d 60. Inclusion of *Capsicum annuum* extract and duration of storage influenced the sensory quality of vacuum-packed cured-smoked chicken fillets to varying degrees. Overall quality of capsicum extract-treated products during shelf storage had higher ratings with increased inclusion level of extract.

Key Words: sensory quality, *Capsicum annuum* extract, vacuum packaging, shelf storage, chicken fillets

302 Breast meat quality parameters affected by wooden breast myopathy according to processing age. E. Oviedo-Rondón, H. Cordova-Noboa, I. Cárdenas-García*, A. Sarsour, V. San Martin, L. Gross, S. Alvarez, I. Martinez, D. Sapcota, D. Lopez, J. Cifuentes, C. Florez, F. Tovar, and J. Barnes, *North Carolina State University, Raleigh, NC.*

Wooden breast (WB) is a myopathy that affects meat quality traits in modern broilers and it has been associated with an increased BW gain and high breast meat yield. Although several studies revealed the negative effects of this disorder on meat characteristics, there is limited information comparing its effects at different slaughtering age. Therefore, the aim of this set of studies was to evaluate the effects of WB myopathy on breast meat quality traits (drip and cook loss, pH, color, and shear force) at 35, 42, 51, 55, and 61 d. At processing day, live BW, carcass yield and breast fillet samples were obtained. Subsequently, samples were subjected to palpation and a WB score was given associated with muscle hardness (1 = none, 2 = mild, 3 = medium, and 4 = severe). Each slaughter age was analyzed separately and individual breast fillets samples were considered as the experimental unit for all the analyses. Data were analyzed using one-way ANOVA and mean separation was done by Tukey's test. A decision tree analysis was conducted to classify WB scores according to BW, carcass and breast yield. At 35 d, the highest ($P < 0.05$) pH values at 6 and 24h post-slaughter (6.07 and 6.12, respectively) were observed in samples affected with severe WB as compared with samples with lower WB severity scores. In addition, weak positive correlations between WB with live BW and breast meat yield were observed ($P < 0.01$; $r = 0.24$, and 0.22, respectively). Results from broiler slaughter at 42 and 51 d, showed ($P < 0.05$) higher pH (6 and 24h post-slaughter), and colorimeter values L* and b* in fillets with severe WB compared with samples with no WB. Additionally, greater drip and cook loss, and shear force ($P < 0.01$) in samples affected with severe WB were observed when compared with samples with no WB at 51 d. Moreover, a moderate correlation ($r = 0.34$; $P < 0.01$) was detected between WB severity with L* value at this age. At 55 d, breast fillets with severe WB had greater drip and cook loss, pH (t = 24h), L*, and b* values as compared with samples with no WB (score 1). Results obtained at 61 d showed a weak positive correlation between WB severity score and breast meat yield ($r = 0.19$; $P < 0.05$). In addition, severe WB (score 4) resulted in higher ($P < 0.05$) shear force, pH, and b* values compared with samples without WB. Decision tree analysis estimated WB higher than score 3 when live BW and breast meat yield differed in ~130 g and 0.3% points among broilers at 61d. In conclusion, WB affected breast meat quality traits at different slaughter age and findings observed in these 5 experiments suggested that WB was not strongly associated with higher BW and meat yield, but probably there is a threshold effect that may be associated with severe WB.

Key Words: wooden breast, broiler, meat quality, slaughter age, live weight

303 Dynamics of antiviral response to Newcastle disease virus (NDV) and heat stress in chicken spleen: RNA-seq in two distinct genetic lines. K. Tracy[2], P. Saelao[2], Y. Wang[2], R. Gallardo[2], S. Lamont[1], and H. Zhou*[2], *[1]Iowa State University, Ames, IA, [2]University of California, Davis, Davis, CA.*

Newcastle disease virus (NDV) poses a major threat in developing countries where NDV vaccination for small chicken flocks is prohibitively expensive and logistically challenging. Discovering determinants of genetic resistance to NDV and other viral pathogens in poultry is an attractive vaccine alternative for areas where the virus is endemic. We therefore investigated the influence of genetics on the progression of NDV infection under heat stress using 2 highly inbred lines that differ in their resistance to NDV. We analyzed the splenic transcriptomes from birds at 2, 6, and 10 d post-infection (DPI) under heat-stress and compared these responses between lines and to birds without treatment. After aligning paired-end RNA-seq results to the chicken genome, we calculated differential gene expression between groups. Gene ontology and pathway analysis of these differentially expressed genes were used to interpret the characteristics of the response to treatment for each line and DPI. Statistical significance was determined using a quasi-likelihood F test for differential gene expression and Fisher's exact test for gene ontology enrichment and pathway analysis. Based on significant predicted pathways and associated functions ($P < 0.001$) of differentially expressed genes ($P < 0.05$), we found that the more resistant Fayoumi birds already display signatures of a resolving antiviral response in the spleen at 2 DPI, followed by no further response at 6 or 10 DPI. The more susceptible Leghorn birds, however, still appear to be upregulating an innate antiviral response at 2 DPI. Although this response does not continue at 6 DPI, at 10 DPI, over half of all analyzed genes are differentially expressed ($P < 0.05$) between control and treated birds, and there are signatures of a dramatic shut down of mitochondrial function ($P < 0.001$). This is consistent with a strong antiviral response and indicates that viral infection under the adverse condition of heat stress may take a larger toll on the less resistant birds. These results suggest that under heat stress, the more genetically resistant birds quickly control viral infection and return to a normal transcriptional profile in the spleen. In contrast, the less resistant birds display a more prolonged response and larger disturbance to normal tissue function.

Key Words: Newcastle disease virus, RNA-seq, antiviral response, heat stress, disease resistance

304 Complete genome sequencing and pathobiological characterization of a virulent strain of Newcastle disease virus isolated from migratory Anseriformes (*Anas platyrhynchos*). M. Z. Shabbir*, A. ul-Rahman, and T. Yaqub, *University of Veterianry and Animal Sciences, Lahore, Pakistan.*

Newcastle disease virus (NDV) is a prototype avian paramyxovirus serotype-1 that causes an economically devastating disease in multiple avian species around the globe. During the recent years, several virulent strains have been isolated from asymptomatic wild and migratory birds worldwide. However, a little is known of their genetic characteristics and potential role in disease transmission and pathology to commercial chicken. We determined genetic, biologic and pathologic characterization of a virulent strain of NDV isolated from mallard (*Anas platyrhynchos*) during 2015–16. Complete genome sequencing was performed through reverse transcriptase polymerase chain reaction using 26 pairs of overlapping primers. Embryo infectious dose (EID_{50}) and mean death time (MDT) were determined by inoculation of isolate to 9-d old embryonated chicken eggs through chorio-allantoic sac route. Transmission and pathology investigation was made by exposure of 100 EID_{50}/mL of isolate to immunologically naïve poultry birds (n = 20) followed by observing disease/clinical symptoms, histopathology and shedding into the environment. Biologically, the isolate revealed to be highly infectious with an EID_{50} ($10^{8.4}$/100 µL) and mean death time (<48 h). Global phylodynamics and evolutionary analysis of the complete genome (15,192bp) of Mallard-I-UVAS-Pak-2016 (KY967611) demonstrated its clustering into lineage 5 (genotype VII). Similar to typical virulent strains of NDVs, the genome encoded for polybasic residue ($_{112}$RRQKR°F$_{117}$) at the fusion protein cleavage motif. The deduced residue analysis for both surface glycoproteins (the fusion and hemagglutinin proteins) revealed important substitutions in the functional and structural domains distinct from known representative strains of each genotype of NDV including the vaccine strains. Clinico-pathological investigations in domesticated chickens indicated its potential to transmit and caused systemic infection involving respiratory and gastro-intestinal tract along with shedding into the environment. Collectively the biologic, pathologic and genomic assessment of study isolate highlight the potential of asymptomatic mallard in carrying virulent NDVs that is genetically divergent and transmissible to commercial poultry and thus, requires necessary intervention for continued surveillance and disease control.

Key Words: Newcastle disease virus, virulent NDVs, Mallard, genotype VI, commercial poultry

305 Role of β-glucan in enhancement of efficacy of vaccination against Newcastle in broilers. R. Gardinal*[1], F. O. R. Filho[1], A. M. Rocha[1], C. A. F. Oliveira[1], J. F. A. Koch[1], B. Mazzer[1], and W. M. Q. Filho[2], *[1]Department of Research and Development, Biorigin Company, Lençóis Paulista, SP, Brazil, [2]University of Sao Paulo, São Paulo, SP, Brazil.*

β-Glucans have been extensively studied for their immunological and other biological effects. The objective of this study was to determine the effect of purified β-glucan supplementation on antibody production during Newcastle disease virus vaccination and performance in broilers. The study was performed at University of São Paulo, Brazil. A total of 220 broilers, one-day-old was housed following a completely randomized design with 2 treatments (1- Control, no supplementation; 2- β-glucan, Macrogard with 69.5% β-glucans, Biorigin, Brazil) and 10 replicates with 22 birds each (density: 10 birds/m^2). Animals were fed with 10 mg of Macrogard kg^{-1} of body weight during 14 d. The animals were immunized via ocular route against Newcastle disease virus (Cevac VitaPest L – CEVA) with primary immunization at 1 d and secondary immunization at 14 d of old. Up to 42 d of age, blood samples were weekly collected from 2 vaccinated and other non-vaccinated broilers from each replicate (10 birds from each treatment). Antibody titers against Newcastle vaccine were measured with ELISA. Feed intake (FI) and body weight (BW) were recorded weekly and feed conversion ratio (FRC) was calculated. Data were analyzed by ANOVA using the PROC MIXED of SAS 9.1 with fixed dietary effect, time effect, interaction between diet and time. Although there were no differences ($P > 0.05$) among the experimental groups in FI, BW and FCR parameters, numerical trends were observed in FI, BW and FCR parameters. Broilers in the MacroGard group demonstrated lower FI and FCR ($P < 0.10$)

besides higher BW ($P < 0.10$) compared control group. The broilers supplemented with β-glucan demonstrated higher antibody titers ($P < 0.05$) during 2 consecutive weeks after the second Newcastle vaccination (at 21 and 28 experimental days) compared with control group. In conclusion, β-glucan supplementation enhances antibody production against Newcastle vaccination and can improve performance of broilers.

Key Words: chicken, health, immunology, prebiotic

306 Can heterophil size indicate a left-shift? I. Observations of atypical duck cells. P. Cotter*, *Cotter Laboratory, Arlington, MA.*

Heterophils may be differentiated from other granulocytes by their dye affinity. However, as granules of both heterophils and eosinophils stain red with standard dyes some misclassification is possible. Their nuclear lobe number estimates age (Arneth Index). An average of ~2.5 describes intermediate ages. Averages of ~2.3 are younger cells indicating "left-shifts." Populations with values of ~2.7 are older ("right shifts"). When applied to poultry, it is assumed heterophils are a population of standard size (diameter, D ~10 μm). However, this is not usually the case. The objective is a description of circulating heterophils of ducks sorted by tincturial classes and size. The method is by a light microscopic examination (Olympus CX-41) of Wright stained venous blood obtained from commercial ducks at ages between 4 – 53 wk. Cells were sorted by duplicate standard differential counts (SDC, 2 × 200 cells) made at 40x magnification and photographed at 100x (oil) magnification. Cell diameters were measured (μm) using Infinity Analyze Software Release 6.5. Average within sample cell size differences were tested by one-way ANOVA with significance at $P < 0.05$. The results indicate multiple heterophil types rather than a single class. Tinctorial types are classic (HC), typical (HT), and variants (HV). HCs have red spindle shaped (fusiform) granules sometimes with a central body. HT granules stain very faintly resembling mammalian neutrophils. HV granules are orange spheres sometimes restricted to one side of the nucleus. SDCs of (n = 31, 4 - 53 woa) commercial ducks gave ~12,120 leukocytes. Standard size heterophils composing 5,800 of the total cells (~43%) were sorted into the 3 tinctorial types as 5,210 HT (~89%), 572 HC (~9.8%), and 9 HV (~0.15%). The average diameters of dwarfs were ~6 μm or ~67% smaller than standards. The nuclear lobe number of dwarf heterophils ranged from 1 to 4 was the same as for standard size cells. Therefore, dwarfs and standard size cells appear to be of similar ages by the Arneth criteria. However, dwarfs are likely a novel form of atypia and may represent an alternate form of a left-shift. Therefore, their presence of dwarfs among standard size cells is indicative of inflammatory reactions. Conclusions: The significance relates to recognizing multiple heterophil tinctorial types, and dwarfs, as indications of atypia and inflammation or infection. Further dwarf heterophils have a direct bearing on the interpretation of H/L ratios a widely used method for determining stress. Thus, the recognition of heterophil tinctorial variants and dwarf types is important in the study of the response to infection, the estimation of stress by blood data, and the establishment of welfare status.

Key Words: heterophil, dwarf cell, stress, welfare, atypia

307 *Bacillus subtilis* 29784 modulates inflammatory responses in a CaCo-2 cell model of induced inflammation. L. Rhayat[1], M. Maresca[2], C. Deschamps*[1], V. Jacquier[1], D. Prévéraud[1], E. Devillard[1], and E. Erik[1], *[1]Adisseo France, Malicorne, France, [2]Aix-Marseille University, CNRS, Marseille, France.*

Birds constantly face different exogenous challenges, through exposure to pathogens, toxins and dietary stresses leading to digestive disorders and thus, impaired performance. Inflammation can be induced in response to stress and must be contained to avoid decrease of performance. Probiotics could have immune-modulating properties and could thus be used to maintain an optimal digestive health. However, as we have shown in a previous study, their effects are likely to be strain dependent, The objective of the present study was to investigate the impact of 3 Bacillus-based probiotics, *Bacillus subtilis* A (Bs A), *B. subtilis* B (Bs B) and *B. subtilis* 29784 (Bs 29784), on epithelial cells, in vitro, using different types of pro-inflammatory stimuli. A human intestinal epithelial cell model (Caco-2) was used to evaluate the ability of these *B. subtilis* strains to modulate inflammation. Vegetative cells of each strain, or epigallocatechin gallate (EGCG) as a control anti-inflammatory substance, were applied to a 14 days differentiated Caco-2 cells monolayer established in a Transwell system. Caco-2 cells were then exposed, or not, to different inflammatory stimuli, either IL-1β, flagella from *Escherichia coli* (Fla) or deoxynivalenol (DON). Transepithelial electrical resistance (TEER) and IL-8 production were then monitored as indicators of intestinal permeability and inflammation, respectively. Not all bacilli showed the same effect on TEER. Regardless of the condition, Bs A appeared to have a negative impact on TEER ($P < 0.01$). Conversely, Bs B and Bs 29784 were able to significantly ($P < 0.01$) improve the TEER, and the strongest effect was observed for Bs 29784. IL8 production was increased ($P < 0.01$) by all stresses applied and the effects of the *Bacillus* strains were strain dependent. Bs A reduced IL-8 release in IL-1β stimulated condition, but increased it upon Fla and DON inflammation ($P < 0.001$). Bs B reduced IL-8 level in IL-1β and Fla stimulated conditions, but increased it in DON stressed condition ($P < 0.001$). Bs 29784 was the only strain able to impact favorably ($P < 0.001$) intestinal permeability and inflammation indicators in all conditions. The results obtained from this study show that depending on the *Bacillus* strains tested the effects on TEER as well as IL8 are different. *B. subtilis* 29784 was not only able to improve TEER, but also to reduce IL-8 production in all stress conditions tested.

Key Words: *Bacillus*, probiotic, inflammation, Caco-2

308 Unprotected butyrate induces bacterial dysbiosis and inflammation in the distal gastrointestinal tract of broilers. P. Moquet*[1], H. Yakout[2], T. Goossens[3], and R. Kwakkel[1], *[1]Wageningen University, Wageningen, the Netherlands, [2]Nutriad Inc., Hampshire, IL, [3]Nutriad International N.V., Dendermonde, Belgium.*

Dysbiosis, a condition related to an imbalanced gut microbiota composition, can negatively impact broilers health and growth performance. Butyrate (Bt) has been shown to exert multiple effects on host-microbiota interactions in distinct gastrointestinal tract (GIT) segments which may influence the onset of dysbiosis. An experiment was conducted to study the effects of providing Bt to distinct GIT segments on cecal microbiota composition and gut health of broilers. A total of 320 male day-old Ross 308 broilers were randomly assigned to 5 dietary treatment groups, each replicated 8 times: one control group and 4 groups supplemented with 1 g/kg Bt in forms having different Bt release profiles: Un-protected Bt (UP); Tri-butyrin (TB); fat-coated Bt (FCB) and an UP-TB combination. Diets were based on rapeseed meal, corn and wheat and were provided ad libitum. At 22–23 d of age, birds were sacrificed. Cecal contents of 5 birds per pen were analyzed for microbiota composition with Illumina HiSeq analysis of 16S ribosomal RNA gene fragments. Ileal and colonic tissues of one bird per pen were analyzed for gut health markers by RT-qPCR. The part of variation in microbiota composition attributable to the dietary treatments was visualized at both phylum and genus levels by redundancy analysis. Changes in cecal microbiota composition and intestinal gene expression were investigated using permutation tests

for the univariate ANOVA and Mann-Whitney *U* test, respectively. UP, which is mostly active in the crop, proventriculus and gizzard, promoted cecal microbiota dysbiosis (e.g., decreased microbial α diversity and increased *Proteobacteria* relative abundance; $P < 0.05$). This was associated with activation of TLR-4 and inflammation in the distal GIT. Feeding TB, which increases selectively Bt concentration in the small intestine, did not induce bacterial dysbiosis in the ceca per se, and yet promoted inflammation in the distal GIT (higher MyD88 expression; $P < 0.05$). FCB (ADIMIXPrecision), which has a sustained release profile with partial protection of Bt against gastric and enteric absorption, did not significantly modify cecal microbiota composition and did not induce inflammation in the distal GIT ($P > 0.05$). Besides, FCB has been reported elsewhere to modify digestive processes in a manner where it can support nutrient digestibility and growth performance. Observed changes in microbiota composition may be attributable to observed modulatory effect of Bt on the expression of host defense peptides and mucus genes ($P < 0.05$). This study indicates that the effect of dietary Bt are conditioned by the GIT segment wherein the molecule is present.

Key Words: butyrate, dysbiosis, inflammation, release location

309 Dietary supplementation of daidzein improve the immunity in the broiler breeder hens and its molecular mechanism. H. Fan*, Z. Lv, L. Gan, Y. Guo, and B. Song, *State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Bei Jing, China.*

Daidzein (DA) is an isoflavone primarily extracted from soy plants, which has wide range of biological roles. A study was carried out to evaluate the effects of supplementing broiler breeder hens with dietary DA on the immune capacity and the underlying molecular mechanism. A total of 480 breeders were divided into 2 treatment groups that were fed either a control diet (CON) or a DA-supplemented diet (DS, CON+20 mg/kg DA) for 8 weeks. Each treatment was replicated 8 times; each replicate included 30 broiler breeder hens. At 4th and 8th week of experiment, for each replicate, blood sample was collected to detect immunoglobulins (Ig), and lymphocyte cells. At 8th week of experiment, one chicken from each replicate was slaughtered, and liver sample was immediately collected from each replicate to measure transcriptome. The results showed that DA supplementation increased ($P < 0.05$) the ratio of B cells in hens at 4th and 8th week of experiment, and the hens in the DS group had higher ($P < 0.05$) levels of IgA and IgM. Moreover, DA supplementation activated the toll-like receptor signaling pathway, positive regulation of response to stimulus, and positive regulation of MAPK cascade. 1095 lncRNAs were identified between the CON and DS groups of hens, 9 lncRNAs of DS group are significant up-regulated compared with CON, and 22 lncRNAs are down-regulated, these lncRNAs are involved in regulating the toll-like receptor and MAPK signaling pathway.In conclusion, dietary DA supplementation at 20mg/kg of broiler breeder hens can improve the immune function by regulating related lncRNAs.

Key Words: daidzein, transcriptome, immunity, LncRNA, hen

310 Evaluation of containerized water based foam methods of depopulation of caged layer hens. R. Alphin*, E. Benson, and D. Hougentogler, *University of Delaware, Newark, DE.*

During the 2014–2015 highly pathogenic avian influenza outbreak, approximately 48 million birds were depopulated or died due to disease. Approximately 40 million were caged layer hens or pullets. The objective of this study was to evaluate alternative methods for depopulating caged layer hens comparing containerized foams to CO_2 gas. For birds raised in cages, depopulation is a challenge and birds either need to be depopulated in cage or removed from the cage and then depopulated. For birds raised in cages, depopulation is a challenge and birds either need to be depopulated in cage or removed from the cage and then depopulated. This is a particular challenge for caged layer complexes that can house as many as 90,000 to 240,000 birds per house and can have more than 20 houses in a complex. Air aspirating foam nozzles (AFN) and foam generators (FG) were 2 water based foam (WBF) options effectively used for floor reared birds during the 2014 – 2015 outbreak, however, neither can be used with birds housed in cages. As a result, new approaches utilizing containerized foaming should be evaluated. Containerized foaming involves removing birds from the cages and treating them with WBF in a container after removal. Two experiments were conducted. COM data was analyzed in JMP Pro 13.1 for distribution and to compare treatments using a student *t*-test with a connecting letters report. In Experiment 1, 16 individual birds were treated with AFN or CO_2 gas. In Experiment 2, 3 groups of 25 birds per replication were treated with FG or CO_2 gas. Experiments were conducted using a randomized block design using commercial table-egg laying hens treated with either a pre-charged WBF or CO_2 gas treatment. An analytical method using an acceleration sensor to sense motion was used to determine when death occurred in relation to the cessation of the motion (COM). In Experiment 1, COM with foam was slower ($\mu = 175.4$ s, $n = 7$) than CO_2 gas ($\mu = 150.7$ s, $n = 8$), however the differences were not statistically significant. In Experiment 2, COM with foam was faster ($\mu = 306.6$ s, $P = 0.038$) than CO_2 ($\mu = 356.4$ s) gassing with 25 birds at a time. The differences shown in this project are consistent with other comparisons of WBF and CO_2 gassing and document that containerized foaming may be an option for caged birds.

Key Words: depopulation, foam, cage, CO_2, layers

311 Intestinal brush border maturation: The effect of in ovo feeding. N. Reicher*, J. Dayan, and Z. Uni, *Hebrew University, Rehovot, Israel.*

The intestinal brush border region is an essential site of nutrient digestion and absorption. It is comprised of microvilli that protrude from enterocyte apical membranes and contain digestive enzymes and nutrient transporters. This work aimed to examine microvilli development pre- and post-hatch and study the effects of in ovo feeding of L-glutamine on microvilli maturation in broiler embryos and hatchlings, by morphological and molecular characteristics. L-glutamine functions as an enterocyte growth factor and was therefore chosen for this study.The experiment consisted of IOF treatments to Cobb500 eggs (n=120) at E17 as follows: 0.6 ml of 1% l-glutamine in 0.4% NaCl (IOF-Gln), 0.6 ml of 0.75% NaCl (IOF-NaCl) and no IOF treatment (control). Jejunum sections of the small intestine from all groups were sampled at pre- and post-hatch periods (E17, E19, DOH, D3, D7, D10) for microvilli measurements by scanning electron microscopy (SEM) and for analyzing expression of genes encoding microvilli core bundling protein (Villin), microvilli stabilizing protein (Ezrin) and microvilli peptide transporter (PepT1) by qPCR. Results showed that short microvilli are evident at E17, prior to villi formation. Microvilli were longer and more uniform at DOH and increased their length by 51% at D3. Significant differences ($P \leq 0.05$) in Villin, Ezrin and PepT1 expression were found between ages: Villin, Ezrin and PepT1 expression increased significantly from E17 to DOH, while post-hatch, Ezrin expression gradually decreased from DOH to D10 and PepT1 expression was significantly lower at D3 and D10 compared to DOH and D7. In the IOF-Gln group at DOH, microvilli were 37% longer than in IOF-NaCl and control groups. Ezrin and PepT1 expression was significantly influenced by IOF at DOH and D10 ($P \leq 0.05$): At DOH, expression of Ezrin and PepT1 was significantly higher in IOF-treatment groups, compared to the control group. In the IOF-Gln group at D10, both genes exhibited higher expression compared to the IOF-NaCl and control groups. There were no significant differences in Villin expression between treatment groups. In conclusion, IOF of L-glutamine enhances maturation processes of the intestinal brush border by microvilli elongation and upregulation of genes involved in microvilli stabilization and nutrient transport. This increases absorptive potential, a highly important factor for intestinal functionality during the first days post-hatch, a critical period for broiler production.

Key Words: in ovo feeding, microvilli, scanning electron microscopy, gene expression

312 Effect of omega-6:omega-3 fatty acid ratios in turkey starter diets on pectoralis major muscle growth. D. Clark*[1], S. Jacobi[1], R. Dilger[2], and S. Velleman[1], *[1]The Ohio State University, Wooster, OH, [2]University of Illinois at Urbana-Champaign, Urbana, IL.*

Omega-3 PUFAs have anti-inflammatory properties which have been shown to improve gastrointestinal health and the immune response when increased in the diet of young chicks. Satellite cells, an adult myogenic stem cell population, are most active at this immediate posthatch period and are particularly sensitive to environmental and nutritional cues. Since, satellite cells are the only cell type with the ability to fuse with existing muscle fibers and increase posthatch hypertrophic growth, dietary recommendations must also consider effects on satellite cell function and long-term effects on posthatch growth. Thus, the objective of this study was to evaluate the effects of omega-6:omega-3 fatty acid ratios in starter diets of turkeys on pectoralis major (p. major) muscle growth. Immediately following hatch, commercial turkey poults (n = 120) were placed in 20 pens (5 pens per treatment) and fed one of 4 diets with calculated omega-6:omega-3 ratios of 38.2:1 (S1, control), 17.7:1 (S2), 8.2:1 (S3), or 3.8:1 (S4). The omega-6:omega-3 ratio was reduced in the dietary treatments by replacing a predominately corn oil blend with a soy/corn/menhaden fish oil blend. Total dietary saturated, monounsaturated, and polyunsaturated fatty acids were included in all diets at an average level of 2.2% (±0.05), 2.7% (±0.05), and 3.7% (±0.1), respectively. Poults were fed dietary treatments for 2 weeks after hatch and then all poults were fed the control diet for an additional 2 weeks. Data were analyzed as a one-way ANOVA in SAS and differences were considered different at $P \leq 0.05$. Feed intake and efficiency were not statistically different ($P \geq 0.14$) for any of the dietary treatments. Body weight was not different ($P \geq 0.11$) at 1, 2, and 3 wks of age; however, at 4 wks turkeys fed the S3 diet for the first 2 weeks after hatch were significantly heavier ($P < 0.01$) than those fed the S1 and S2 diets. Additionally, those fed the diet with the lowest omega:6-omega-3 ratio (S4) were heavier ($P = 0.02$) than those fed the S2 diet during the first 2 weeks after hatch. At 2 weeks of age, the poults fed the control diet had heavier p. major muscle weight, both as a percentage of body weight ($P < 0.01$) and absolute weight ($P \leq 0.01$) compared with all other dietary treatments. However, by 4 weeks of age there were no differences ($P \geq 0.19$) in p. major weights between dietary treatments. Together these data demonstrate that altering the omega-6:omega-3 fatty acid ratio in starter poult diets may affect p. major muscle growth, which could have long-lasting implications on meat quality by altering the myofiber structure of the p. major muscle.

Key Words: omega-3 fatty acid, pectoralis major, poult, starter diet, turkey

313 Dietary protein and dexamethasone influence gut permeability and gene expression of selected tight junction proteins and sodium-dependent glucose transporter in the ileum of broiler chickens. R. Barekatain*[1], G. Nattrass[1], S. Kitessa[1], K. Chousalkar[2], and S. Gilani[2], *[1]South Australian Research and Development Institute, Roseworthy, South Australia, Australia, [2]University of Adelaide, Roseworthy, South Australia, Australia.*

Feed cost, environmental effects, and availability of quality food protein sources have prompted research into the use of use of low protein (LP) diets. However, little is known about the effect of reducing dietary protein and in general concentration of amino acids on intestinal barrier function and permeability. This study was conducted using male Ross 308 birds to determine the effect of 3 experimental diets: LP (17% CP) fortified with essential amino acids, standard protein (SP, 20.2%) and high protein (HP, 22%). Dexamethasone (DEX), a synthetic glucocorticoid, was used to induce leaky gut. Diets were formulated to meet the Ross 308 specifications to have a similar level of essential amino acid in LP and SP diets, while 10% above the recommended Ross specifications was used for HP diets. Birds were reared on the same starter diet until d 9 of age and then assigned to the 3 experimental grower diets. On d 13, 72 birds were placed in individual cages (24 replicates per diet). Half of the birds (12 birds per diet) were injected intramuscularly with DEX (0.5mg/kg) on d 14, 16 and 20 while the remaining 36 birds received sham injections. The fluorescein isothiocyanate dextran (FITC-d) was utilized to test permeability on d 21 followed by euthanizing birds for

tissue collection. A quantitative PCR was conducted on ileal tissues for mRNA expression of selected tight junction proteins (Claudin-1, Claudin-3, JAM-2, and ZO-2) and sodium-dependent glucose transporter-1 (SGLT-1). The main effects of DEX and diet and their interaction were assessed using a 2-way ANOVA and treatment means were separated by Fisher's LSD test ($P \leq 0.05$ as significant). With no interaction, LP fed birds had a higher ($P = 0.02$) level of FITC-d in serum indicating a more permeable intestine only when compared with HP. DEX increased ($P < 0.001$) FITC-d concentration in all dietary treatments. There was no interaction between DEX and diets for the selected genes except for JAM-2. HP diets increased the abundance of JAM-2 in unchallenged birds whereas the reverse was observed compared with LP in DEX injected birds. Expression of Claudin-1 was reduced ($P < 0.05$) in HP treatment independent of DEX. Birds fed LP had less ZO2 expression ($P < 0.01$) in comparison with SP, but not HP. DEX increased the expression of Claudin-3 ($P < 0.001$) and ZO-2 ($P < 0.05$) and reduced Claudin-1 ($P < 0.05$) and JAM-2 ($P < 0.001$). Expression of SGLT-1 was upregulated ($P < 0.05$) in LP fed group. It is concluded that LP compared with HP may lead to a higher intestinal permeability and differentially expressed ZO-2 and SGLT-1 in the ileum. DEX found to have a profound independent effect on intestinal barrier function evidenced by expression of tight junction genes.

Key Words: intestinal barrier function, permeability, low protein, amino acid, glucocorticoid

315 Influence of the length of the feed restriction period on the anticipatory feeding behavior and the development of the proximal part of the gastrointestinal tract in broilers. C. Amán, G. Fondevila, J. L. Archs*, L. Cámara, and G. Mateos, *UPM, Madrid, Madrid, Spain.*

The effects of the length of the feed restriction period on the anticipatory feeding behavior and the development of the proximal part of the gastrointestinal tract (GIT) were evaluated in broilers from 7 to 19 d of age. The experimental design was completely randomized with 4 different length of the fasting period: 0, 4, 6, and 8 h per day. Fasting conditions consisted in restricting the access of the birds to the feeders. Each treatment was replicated 6 times and the experimental unit was a cage with 13 chicks. All birds received a common mash diet based on wheat corn and SBM throughout the experiment. At 7, 10, 13, 16, and 19 d of age, immediately before the start of the corresponding fasting period, 2 birds per cage chosen at random were euthanized by asphyxia using a CO_2 atmosphere, weighed individually, and used to study the development of the crop and the gizzard. The full and empty weights, the pH, and the content and moisture of the digesta of both organs were recorded. The anticipatory feeding behavior of the birds was measured indirectly by studying their capability to fill the crop immediately before the start of the fasting period. In addition, the number of colonies of Lactobacillus spp. was determined in the crop at 19 d of age. Data on crop and gizzard traits were using the mixed procedure of SAS analyzed with length of the feed restriction period and age of the bird as main effects. Additionally, treatment sum of squares for the fasting length and the age of the birds was partitioned into linear (L) and quadratic (Q) effects. Age of the birds affected significantly linear effect all variables studied. Prior to the start of the fasting period, the absolute (g) and relative (% BW) weight of the full and empty crop, increased (L: $P \leq 0.001$) and the fresh and dried content of the digesta (L: $P \leq 0.001$) increased as the length of the fasting period increased. Length of the fasting period did not affect any gizzard trait, including pH, at any age. The Lactobacillus spp. colony count was not affected by fasting. In summary, an stimulatory effect of fasting on anticipatory feeding behavior and crop development was detected. The data suggest that limiting the access of broilers to the feed for 6 h, or more improves the development of the proximal part of the GIT in broilers from 7 to 19 d of age.

Key Words: broiler, crop development, feed restriction, gizzard development, organ pH

316 The effect of tea polyphenol on production performance and egg quality of laying hens fed long-term storage corn. L. Zhou*[1], X. Ding[1], J. Wang[3], S. Bai[1], Q. Zeng[1], Z. Su[1], and Y. Xuan[2], *[1]Animal Nutrition, Wenjiang,Chengdu, Sichuan, China, [2]Institute of Animal Nutrition, Key Laboratory for Animal Disease-Resistance Nutrition of China Ministry of Education, Sichuan Agricultural University, Chengdu, China, [3]Sichuan Agricultural University, Chengdu, China.*

Tea polyphenol is a natural antioxidant that can improve the antioxidant status of eggs. This study examined the effects of dietary supplementation of tea polyphenol (TPP), on production performance, egg quality, serum parameters and yolk fatty acid profile in laying hens fed long-term storage corn (SC, storage up to 4 yr at room temperature in barns). A total of 288 Lohmann commercial laying hens (63-wk-old) were used under a 2 × 4 factorial arrangement with 4 levels of dietary SC (0, 25%, 50%, or 100%), and 2 levels of TPP (o and 600mg/kg) for 8wk. All data were analyzed by 2-way ANOVA, and the main effect together with their interactive was determined in final model. Dietary 100%SC with no TPP decreased ($P < 0.05$) the laying ratios and increased ($P < 0.05$) the feed conversion ratios compared with the control treatment. Dietary 100%SC with no TPP decreased ($P < 0.05$) the Albumen height and yolk index compared with the 100%SC+TPP, 50%SC+TPP, 0%SC+TPP. The addition of TPP significantly increased ($P < 0.05$) the serum total cholesterol, while decreased ($P < 0.05$) the uric acid of laying hens. Dietary SC linearly decreased ($P < 0.01$) the egg yolk color, and trend to linearly decreased the polyunsaturated fatty acids ($P < 0.1$) and n-3 polyunsaturated fatty acids ($P < 0.1$). In conclusion, the dietary 100%SC had adverse effect on production performance and egg quality, which can improved by the addition of TPP, except egg yolk color.

Key Words: laying hen, long-term storage corn, tea polyphenol, production performance, egg quality

317 Effects of the nutritional profile of the pre-laying diet on productive performance and egg quality traits of brown-egg laying hens from 15 to 62 weeks of age. L. Aguirre[1], J. García[2], R. Scappaticcio[2], G. Fondevila[1], and G. Mateos*[1], *[1]UPM, Madrid, Madrid, Spain, [2]Camar Agroalimentaria S. L., Toledo, Spain.*

In total, 540 Hy Line Brown-egg laying hens were used to study the effect of the pre-laying diet on productive performance from 18 to 62 wk of age. All the pullets received a common rearing feeding program from hatch to 15 wk of age. From 15 to 18 wk of age, the pullets were divided at random into 3 groups and fed 3 different diets: a) a rearing pullet diet (PUL, 2,700 kcal AMEn/kg, 0.61% SID Lys, and 1% Ca), b) a pre-laying diet (PRE, 2,750 kcal AMEn/kg, 0.78% SID Lys, and 2.5% Ca), and c) a laying hen diet (LAY, 2,750 kcal AMEn/kg, 0.78% SID Lys, and 3.9% Ca). From 18 to 62 wk of age, all hens received a common SBM-corn and wheat diet. The experimental design was completely randomized with 3 treatments and 18 replicates of 10 hens each per treatment. From 15 to 18 wk of age, average egg production was low for all treatments (<5%) and none of the variables studied, except ADFI which was higher for the PUL diet ($P \leq 0.001$), was affected by diet. Hen performance (egg production, egg mass, FCR,

and mortality) was controlled by replicate and the data were analyzed by period (4 wk) and cumulatively (18 to 62 wk of age). Egg quality, including yolk pigmentation, Haugh units, shell thickness, shell strength, and absolute (g) and relative (% egg weight) weight of the shell, were determined by replicate in 6 eggs chosen at random for the last 2 d of each of the 11 experimental periods. Percentage of broken and shell-less eggs were determined in all eggs produced. Data were analyzed as a completely randomized design with feeding strategy as main effect using the MIXED procedure of SAS. The Tukey test was used to make pairwise comparisons to separate treatment means. Potential interactions between dietary treatment and period was studied using the repeated measurements analysis. From 18 to 62 wk of age, feeding strategy did not affect hen performance or any of the egg quality traits studied. Data on egg weight and on all shell quality variables measured, favored in all the 11 periods the use of the LAY diet compared with the use of the other 2 pre-lay diets. The differences detected, however, were significant only in few of periods. In fact, no differences among treatments were detected for the entire experiment. The reason for the limited effect of the pre-laying diet on the shell quality variables studied could be that few hens were laying eggs before 18 wk of age, when all the birds were fed a common LAY diet. In summary, hen performance and egg quality from 18 to 62 wk of age were not affected by the type of feed supplied from 15 to 18 wk of age, although some egg quality data support the use of the LAY diet in the pre-laying period from 15 to 18 wk of age.

Key Words: laying hen, nutritional strategies, pre-laying, productive performance

318 Influence of feed form on performance and development of the proximal part of the gastrointestinal tract of broiler strains with different growth potential. G. Fondevila*, L. Cámara, J. L. Archs, and G. Mateos, *UPM, Madrid, Madrid, Spain.*

The effects of feed form on growth performance and on the development of the proximal part of the gastrointestinal tract (GIT) of 2 broiler strains were studied from 1 to 22 d of age. The experimental design was completely randomized with 4 treatments arranged as a 2 × 2 factorial with feed form (mash vs. pellet) and bird strain (Ross 308 vs. Hubbard JV) as main effects. A common corn-soybean meal diet (2,970 kcal AMEn/kg and 1.30% SID Lys) presented as mash (GMD ± GSD = 713 µm ± 1.73) or pellets (2 mm Φ) was used. Each treatment was replicated 6 times and the experimental unit was a cage with 9 chicks. BW and ADFI were recorded by replicate at 7, 17, and 22 d of age and FCR was calculated from these data. At the same ages, 2 birds per cage chosen at random were weighed individually, euthanized by asphyxiation in CO_2 atmosphere, and used to study the development of the proximal part of the GIT. Main effects of strain, feed form, and age and their interactions on growth performance and GIT traits were analyzed using the MIXED procedure of SAS. Ross birds were heavier and had better FCR ($P \leq 0.001$) than Hubbard birds. Pelleting increased ADG and improved FCR ($P \leq 0.001$), benefits that were more evident for the Ross than for the Hubbard birds ($P \leq 0.001$ for the interaction). Regardless of strain and age, pelleting increased the relative weight (RW; % BW) of the full crop and gizzard, as well as the fresh and dried contents of the crop ($P \leq 0.001$). In absolute terms (g), the empty crop and gizzard were heavier in the Ross than in the Hubbard birds at all ages ($P \leq 0.001$) but no differences were detected in relative terms. The pH of the crop and gizzard digesta was lower for the Ross than for the Hubbard birds ($P \leq 0.001$). In summary, pelleting improved consistently growth and feed efficiency of broilers from 1 to 22 d of age. Pelleting increased the absolute weight and the digesta content of the crop but decreased the RW of the gizzard and its content. Also, pelleting increased the pH of the gizzard. The data indicate that the effects of pelleting on growth performance and on the development of the proximal part of the GIT were more accentuated in Ross than in Hubbard broilers.

Key Words: broiler strain, crop, feed form, gizzard, organ pH

319 Effects of lysolecithins and hydrocolloids on performance, nutrient digestibility, true metabolizable energy, lipid profile, and carcass quality of broilers. R. Riboty[2], J. Garcia[2], and C. Vilchez*[1], *[1]Universidad Nacional Agraria la Molina, Lima, Peru, [2]Universidad Tecnologica Equinoccial, Quito, Ecuador.*

The objective of the study was to evaluate the effects of the inclusion of lysolecithins and hydrocolloids in a ME deficient-basal diet on performance, nutrient digestibility, true metabolizable energy (TME) content of the diet, lipid profile and carcass quality of broilers raised at 2600 m.a.s.l. 1120 one-day-old Cobb 500 mixed chicks were randomly divided into 28 experimental units with 40 chicks each. Animals of 7 experimental units received, during 42 d, one of the following treatments: T1, Positive control diet; T2, Negative control diet (NC; - 100 kcal/kg ME than that of T1); T3, NC diet + Lysolecithins (1000 ppm) and T4, NC + Hydrocolloids (1000 ppm). A 3-phase (1–14, 15–28 and 29–42 d) restricted mash feeding program was used. Eighty (including a blank group), 7 and 28 (14 male and 14 female) birds per treatment were used to determine nutrient digestibility and TME of the experimental diets, lipid profile and carcass quality, respectively. Statistical significance was evaluated using ANOVA under a Randomized Complete Block Design with Tukey's test for multiple comparisons. The results indicated that feed intake and lipid profile were not influenced ($P > 0.05$) by dietary treatments. However, weight gain and feed conversion of birds that were under T2 were lower ($P < 0.05$) than those of T1 while those corresponding to T3 and T4 were intermediate. The lowest nutrient digestibility and TME content of the experimental diets were observed in birds that received T2 with the highest corresponding to those of T1. Carcass weight was not influenced ($P > 0.05$) by either dietary treatment or sex; however, high ($P < 0.05$) abdominal fat content was observed only in male birds that were fed T1 as compared with the other treatments. In conclusion, birds that were fed either lysolecithins or hydrocolloids, in a ME deficient basal diet, do not perform as well as those that were under the positive control diet suggesting that both additives release energy but less than the estimated 100 kcal/kg.

Key Words: lysolecithin, hydrocolloid, digestibility, emulsifier, metabolizable energy

320 Protein degradation of broiler breeders from the onset of lay through peak production. G. Mullenix*[1], X. Ding[2], J. England[1], K. Hilton[1], M. Schlumbohm[1], and C. Coon[1], *[1]Umiversity of Arkansas, Fayetteville, AR, [2]University of Arkansas, Chengdu, China.*

The objective of the study was to see how protein degradation effects the onset and peak of lay in broiler breeders. Excreta was collected from hens at 28, 31, 35, 37, 39, 41 and 44 wks of age. 3-methylhistidine (3-mh) was extracted from a 24 h excreta collection and measured on an Agilent 7890A gas chromatographer/5975C mass spectrometer. Uric Acid in the excreta was analyzed at peak production, 31 weeks, with a micro plate photometer. 648 broilerized pureline pullets were obtained at 21 wks of age and placed in individual metabolic cages. At 28 wks of age, 90 birds (n = 30 birds/weight group) were allocated into small, medium and large weight groups. All birds were restrictively fed a generic corn/soybean breeder diet throughout the trial. Although the different weight group of birds showed no statistical difference ($P = 0.4191$) in degrada-

tion of 3-mh between the large ≈4188.06g (426.09 ± 37.13 µmol/kg), medium ≈3668.93 (386.18 ± 41.8µmol/kg) and small≈3043.1 (361.77 ± 31.6 µmol/kg) weight groups, there was a linear relationship (R2 = 0.9942). Uric Acid excretion showed no significant relationship to eggs produced per hen per day ($P = 0.9764$), eggs produced per week ($P = 0.3005$) or body weight group ($P = 0.4106$). Protein degradation did significantly increase for all birds from 28 wks (446 ± 53 µmol/kg) to 31 wks (780.72 ± 53 µmol/kg) of age ($P < 0.0001$), before decreasing to 296.58 ± 53 µmol/kg at 44 wks. Total eggs produced per week by hen ($P < 0.0001$) and eggs produced per day by hen ($P < 0.0001$) increased significantly from 28 wk to 31 wk of age; and from 31 wk to peak production at 37 wk of age ($P < 0.0001$). Excreta 3-methylhistindine didn't show a correlation to eggs produced in a week; however when a hen laid more than 1 egg in a 24 h period their 3-methylhistidine increased substantially (280µmol/kg of BW). This data indicates that broiler breeders are maximizing their skeletal protein degradation during the onset of lay to bring themselves into production and there is a linear relationship of 3-mh and BW.

Key Words: 3-methylhistidine, breeder, degradation

321 Influence of dietary energy densities and starch to lipid ratios on broiler performance. S. Liu*[1], P. Chrystal[2], A. Moss[1], and P. Selle[1], *[1]University of Sydney, Brownlow Hill, NSW, Australia, [2]Baiada Poultry Pty Limited, Pendle Hill, NSW, Australia.*

Energy densities and digestible amino acids are considered in tandem in the practical formulation of diets for broiler chickens and essentially energy may be derived from starch, protein and lipid. The impacts of protein and the importance of the protein and energy balance are well documented. However, few studies have compared the relative importance of starch and lipid. Twelve experimental diets in a factorial arrangement with 3 energy levels (11.25, 12.38 and 13.50 MJ/kg) and 4 starch to lipid ratios (14, 12, 7, 4:1 g/g) were offered to 4 replicate cages (6 birds per cage) or a total of 288 male Ross 308 broiler chicks from 7 to 27 d post-hatch. All dietary treatments were formulated to contain similar digestible lysine to AMEn ratios and amino acid profiles. Increasing energy density from 11.25 to 13.50 MJ/kg decreased feed intake by 6.4% (2314 versus 2167 g/bird, $P < 0.001$), increased weight gain by 10.5% (1511 versus 1670 g/bird, $P < 0.001$) and improved FCR by 15.3% (1.533 versus 1.298 g/g, $P < 0.0001$). Widening starch to lipid ratios linearly increased weight gain (r = 0.448, $P = 0.001$) and feed intake (r = 0.509, $P < 0.001$) without influencing FCR ($P > 0.75$). Lipid had a more pronounced impact on feed intake than starch. The protein leverage hypothesis suggests that humans and animals prioritise protein intake when they are forced to select protein intake against that of carbohydrate and lipid derived from nutritionally unbalanced diets. In the present study, protein intake remained constant when the dietary protein to [starch + lipid] ratios increased. This indicates that broiler chickens maintained a constant protein intake of around 450 g/bird regardless of dietary protein concentrations. In contrast, starch intakes increased linearly (r = 0.917; $P < 0.0001$) with its dietary proportions. Lipid intakes increased with dietary lipid to [starch + protein] ratios and reached maximum of 167 g/bird when the ratio equalled 0.198 g/g. This suggests that broiler chickens do not consume to constant starch and lipid intake targets. That lipid intakes plateaud may be due to its negative impact on pellet quality and, in turn, feed intakes of broiler chickens. Given that the protein intake remained constant it becomes important to understand the influence of individual amino acids on growth performance in future research.

Key Words: energy, growth, lipid, starch, protein

322 Influence of bird type and corn particle size on nutrient utilization. M. R. Abdollahi*, A. Mtei, N. Schreurs, and V. Ravindran, *Massey University, Palmerston North, New Zealand.*

The interaction between bird type (broilers and layers) and corn particle size (fine, medium and coarse) on the coefficient of apparent ileal digestibility (CAID) of nutrients was investigated in a 2 by 3 factorial arrangement of treatments. Whole corn was ground in a hammer mill to pass through screen sizes of 2.0, 5.0 and 8.0 mm for fine, medium and coarse grades, respectively. A corn and soybean meal was formulated and mixed for both broilers and layers, using the same batch of ingredients. Titanium dioxide (5.0 g/kg) was included in all diets as an indigestible marker for digestibility measurements. 35-d old male broilers and 59-weeks old layers were used in this study. For each bird type, 108 birds with uniform body weights were assigned to 18 replicates (6 replicates per treatment and 6 birds per replicate). Birds were fasted for 12 h before the introduction of treatment diets, and the diets were offered ad libitum for 7 d before the collection of ileal digesta. After 7 d on treatment diets, all birds were euthanized by intravenous injection of sodium pentobarbitone and ileal digesta was collected from the lower half of the ileum. The data was analyzed by 2-way ANOVA to determine the main effects (bird type and particle size) and their interaction using the General Linear Models procedure of SAS. A bird type by corn particle size interaction was observed ($P < 0.001$) for CAID of dry matter (DM), gross energy (GE) and starch. In broilers, CAID of DM, GE and starch was not influenced ($P > 0.05$) by particle size, while in layers, increasing corn particle size to medium and coarse resulted in higher CAID of these nutrients compared with fine particles. The CAID of nitrogen and fat was greater in broilers than layers ($P < 0.05$) and the opposite was observed ($P < 0.05$) for those of calcium (Ca) and phosphorus (P). Regardless of bird type, coarsely ground diet resulted in similar Ca digestibility to the diet with medium particle size but higher than the diet with fine particles ($P < 0.05$). Overall, the present data showed that broilers had a greater nutrient digestibility than layers, except for Ca and P. Feeding medium and coarse corn particles benefited nutrient digestibility only in the layers, suggesting that digestive system of layers is probably more sensitive to particle size or feed texture than broilers.

Key Words: broiler, layer, corn, particle size, nutrient digestibility

323 Chemical composition, nutritive value, and protein quality of soybean meals from beans produced in different countries: a meta-analytical study. L. Cámara*, C. de Blas, M. Á. Ibáñez, and G. Mateos, *UPM, Madrid, Madrid, Spain.*

Soybean meal (SBM) is the most important source of protein in non-ruminant diets worldwide. The chemical composition, protein quality and nutritive value of commercial SBM depend on numerous factors, including the maturity of the seeds, the conditions of the process used to extract the oil, and the area of production of the original beans. A meta-analytical approach was applied to evaluate the relation between the country of origin of the beans [Argentina (ARG), Brazil (BRA), India (IND) and USA] and the chemical composition and protein quality traits of the SBM. The data set used was obtained from 17 published papers with a total of 1,334 samples of SBM. The manuscripts were scrutinized based on the following criteria: 1) peer reviewed papers published between 2002 and 2017, 2) origin of the beans was clearly stated and documented, 3) origin of the beans was used as main effect, 4) at least 2 different soybean origins were compared in the same trial, and 5) the analytical methods used to determine the composition and protein quality of the SBM were reported. The data were analyzed using a mixed model with origin of the beans as a fixed effect and the study

as a random effect. The data showed that the country of origin of the beans had consistent and significant effects on most of the variables studied, with differences being significant in most cases. Brazilian SBM had more CP, neutral detergent fiber, raffinose, and Fe but less sucrose, stachyose, and K than ARG and USA SBM ($P < 0.001$). Per unit of CP, Lys, Met, Thr, and Cys contents were greater for the USA and ARG meals than for the BRA and IND meals ($P < 0.001$). Also, PDI, KOH solubility and TIA values were lower for the BRA and ARG meals than for the USA and IND meals. The calculated (MJ/kg DM) AMEn for poultry (11.06 vs. 10.95 vs. 10.90) was higher for BRA than for USA and ARG SBM. However, calculated NE (MJ/kg DM) for pigs (9.44 vs. 9.39 vs. 9.37) was higher for USA meals than for BRA and ARG meals. Pearson correlation showed a significant ($P < 0.001$) relation between KOH and TIA (r = 0.886), PDI and TIA (r = 0.712), and PDI and KOH (r = 0.614). In summary, country of origin of the beans affected the chemical composition and nutritive value of the SBM. Nutritionists should be aware of the existing differences and use different matrix values for SBM, according to the origin of the beans, when formulating commercial diets for domestic animals.

Key Words: amino acid profile, chemical analysis, protein quality, soybean origin

324 Using yeast from ethanol production as a protein source in broiler diets on growth and meat yields. Wei Zhai*[1], K. O'Donnell[1], and K. Herrick[2], *[1]Mississippi State University, Mississippi State, MS, [2]POET, Sioux Falls, SD.*

A trial was conducted to evaluate a yeast product (an ethanol production co-product from corn by POET) as a potential protein source in broiler diets on growth performance and meat yield of broiler chickens. A total of 1,520 d-old Ross × 708 male broilers were divided into 8 dietary treatments with 10 replications for each treatment. The birds were fed common diets from d 0 to 9. On d 9, birds were equalized across pens according to their BW, and 15 birds were kept in each pen. Eight experimental diets including 1) Corn-soybean meal (SBM) control diet, 2) Corn-SBM with either 4% DDGS, 3) 4% meat bone meal (MBM), 4–6) 2, 4, 6% POET yeast product, 7) 2% Product A, and 8) 0.2% Product B (both A and B are commercial available yeast products), were fed to the birds after BW equalization. Diets were formulated to contain same ME and digestible lysine, methionine and threonine. Data were analyzed by one-way ANOVA using Proc Mixed of SAS 9.4. Mortality from d 9 to 56 was not affected by any dietary treatments ($P = 0.444$). Feeding various diets did not affect BW on d 21 or 56 ($P = 0.411$, $P = 0.630$). Body weight on d 35 of birds fed MBM, 2, 4, and 6% POET yeast product and BW on d 42 of birds fed MBM and 4% POET yeast product were improved as compared with those fed Corn-SBM control diet ($P = 0.010$, $P = 0.049$). Birds fed MBM and 4% POET yeast exhibited lowest FCR from d 9 to 35 ($P = 0.003$). The 4% and 6% POET yeast inclusion in the feed improved d 42 carcass weights, breast weights, and overall front weights (including wing, breast, and tender), as compared with the Corn-SBM control ($P = 0.006$, 0.021, and 0.010). Most cut part yields of the bids fed 4% and 6% were similar to those of birds fed MBM, or even higher (such as d 42 breast weight of 4% POET). As opposed to the changes on carcass and breast weights, inclusion of 4% and 6% POET yeast had a trend to lower absolute abdominal fat weight ($P = 0.096$) and relative fat to BW ($P = 0.051$), as compared with control diet. On d 56, carcass and main cut parts were not affected by any diets. The main effects during the withdrawal period were on fat. The 4% POET yeast inclusion lowered absolute ($P < 0.0001$) and relative ($P < 0.0001$) fat weights on Day 56. In conclusion, the highest inclusion level of the POET yeast product (6%) tested in this trial is safe for Ross 708 male broilers. The inclusion of POET yeast product exhibited more significant benefits to the birds in early growth stages, especially on d 35 and 42. Moreover, not only the meat production was improved, the fat was decreased, which may indicate that nutrients of those POET diets were utilized more effectively by the birds.

Key Words: broiler, growth, meat yield, protein source, yeast

325 The effect of PB6 and encapsulated butyric acid and zinc on performance of broilers during necrotic enteritis challenges caused by *Clostridium perfringens*. K. Vignale-Pollock*[1], K. Chasser[2], W. Briggs[2], and L. Bielke[2], *[1]Kemin Industries, Inc., Des Moines, A, [2]Ohio State University, Columbus, OH.*

The objective of the current experiment was to evaluate the effects of encapsulated butyric acid and zinc (EBZ) and PB6 on performance and livability of broilers in necrotic enteritis (NE) situations. The experiment was a complete randomized block design arranged in a 2 × 2 factorial design. At day of hatch, Ross 708 male chicks were randomly assigned to one of 4 treatments (12 reps/treatment; 20 birds/rep): control (no additives), PB6 (CLOSTAT, Kemin Industries, Inc.;1 lb/ton), EBZ (ButiPEARL Z, Kemin Industries, Inc.; 1lb/ton) and EBZ (1 lb/ton) + PB6 (1lb/ton). On d 15, all pens were challenged with *Eimeria maxima* M6, then challenged with *Clostridium perfringens* on d 18, 19, 20, and 21. On d 21, 5 birds/pen were scored for NE lesions. The scoring was based on a 0 to 4 scale. Statistical analyses were performed using JMP (SAS Institute, Cary, NC) software. All data were subjected to a 2 × 2 factorial ANOVA with main effect means deemed significantly different at $P \leq 0.05$. No differences were observed in lesion scores and mortality ($P > 0.05$). No interactions were seen for any of the parameters measured ($P > 0.05$). There was a significant PB6 effect for BW, body weight gain and FCR ($P < 0.05$). BW was significantly improved by PB6 at 28 d and 42 d ($P < 0.05$). Body weight gain was significantly improved by PB6 during the 2-week period of challenge and post-challenge (d 21 – 35; $P < 0.05$) and at 0–42 d ($P < 0.05$). PB6 significantly improved FCR at 0 - 35 d by 2 points when compared with control ($P < 0.05$). There was a significant EBZ effect for BW, BWG and FCR. EBZ significantly improved BW at 28 d and 42 d ($P < 0.05$). BWG was significantly improved by EBZ during the 2-week period of challenge and post-challenge (d 21 – 35; $P < 0.05$) and at 0–42 d ($P < 0.05$). EBZ significantly improved FCR at 0 – 35 d and at 0 – 42 d by 4 and 5 points, respectively, when compared with the control ($P < 0.05$). PB6 and EBZ were effective at alleviating performance losses associated with the disease.

Key Words: butyric acid, necrotic enteritis, performance, broilers, zinc

326 Encapsulated sodium formate improves performance and intestinal health in broilers challenged with necrotic enteritis. J. Rubach[1], V. Mani*[1], P. Mannina[1], B. Lumpkins[2], G. Mathis[2], and M. Poss[1], *[1]Kemin Industries, Des Moines, IA, [2]Southern Poultry Research Inc., Athens, GA.*

Necrotic enteritis (NE) has a significant economic burden on the poultry industry. *Clostridium perfringens*, the causative organism of NE, primarily affects the intestine, leading to compromised intestinal integrity and poor gut health. The NE burden is further confounded by concurrent infection with coccidiosis, significantly reducing the performance of the birds. Formic acid and its salts are widely used as gut acidifiers in swine, but the use of formic acid in the poultry industry is limited. To determine the efficacy of sodium formate, in mitigating the negative effects associated with NE, a novel formulation of encapsulated sodium formate (ESF, FORMYL Na, Kemin Industries) was tested in a broiler NE challenge trial. In the 42-d pen trial, 3000 d of hatch Cobb × Cobb 500 chicks were assigned to 60 pens, divided into 6 treatments with 10 replications per treatment (50 birds/pen, 500 birds/trt). The birds were vaccinated with Coccivac-B52 (Merck Animal Health) on day of hatch and were challenged through feed with *Clostridium perfringens* on d 19, 20 and 21. Three controls included, non-challenged and non-medicated (NCNM), challenged and non-medicated (CNM), challenged and medicated with 60 g/ton of bacitracin (BMD). Three doses of ESF (5, 10 and 20 lb/ton) were tested. On d21 and 35, intestines of 3 birds randomly selected from each pen were scored for necrotic enteritis lesions and coccidial oocysts were measured in the feces. Results were analyzed using one-way ANOVA with the individual treatments compared by *t*-test. On d42 the average feed intake for the BMD, 5 lb/ton ESF and 10 lb/ton ESF treatments were statistically greater than the CNM group ($P < 0.05$). The average weight gain on d42 showed that the BMD and 5 lb/ton ESF treatments were statistically similar to the NCNM group ($P > 0.05$) and significantly greater than the CNM group ($P < 0.05$). The d42 FCR showed all 3 ESF treatments were statistically improved compared with the CNM group ($P < 0.05$) and statistically equal to the BMD group ($P > 0.05$). Necrotic enteritis lesion scores on d21 showed that the BMD and ESF treatment groups all had significantly lower lesion scores than the CNM group. The total mortality due to NE was significantly reduced for the BMD and ESF treatment groups, as compared with the CNM group ($P < 0.05$). Results from this study indicate that encapsulated sodium formate can alleviate NE caused by *Clostridium perfringens*, and that encapsulated sodium formate can be an effective alternative to antibiotics.

Key Words: necrotic enteritis, coccidiosis, formate, intestine, poultry

328 Combatting the most occurring mycotoxin in South America: Fumonisins. M. Gallissot*, J. Laurain, and M. A. Rodriguez, *Olmix, Brehan, France.*

A recent survey highlighted that, in South America, 94.4% of the feedingstuff samples analyzed between 2013 and 2016 were contaminated with fumonisins, making it the most occurring mycotoxin in the area, and representing a significant threat for animal performance. The objective of the study was to evaluate the capacity of an algae-clay based product (MT.X+) to manage fumonisin risk in poultry production. Three trials were implemented. The first trial, conducted by the Samitec Institute (Brazil), tested the capacity of this product to decrease the effects of an acute fumonisin contamination (100 ppm) under experimental conditions. The 2 other trials, conducted in field conditions, tested the capacity of the product to counteract fumonisin effects on breeding hens (n = 50,500) and broiler chickens (n = 308,700) in a context of natural fumonisin contamination (from 850 to 3,500 ppb). The results of the experimental study showed that the product significantly improved performance (feed intake and growth), reduced by the contamination in fumonisins. Clinical biochemistry and liver parameters were also significantly improved. In the field, the product increased the laying rate of breeding hens (+6%, $P = 0.014$), leading to an improved productivity of the farm. In broiler chickens, the product increased the production efficiency factor (PEF) by 10% and the return over feed cost by 36% compared with control. This series of trials confirmed that fumonisin contamination has a negative impact on poultry performance. The use of a specific algae-clay based product succeeded in preventing this impact on performance and productivity, both in experimental and commercial conditions.

Key Words: mycotoxin, fumonisin, algo-clay

329 External and internal quality of eggs from quail hens fed diets containing diferent levels of alkaloid-free coca leaf extract. E. Bernuy and C. Vilchez*, *Universidad Nacional Agraria la Molina, Lima, Peru.*

The objective of this experiment was to determine the internal and external quality of eggs from Japanese quail fed on diets that contained different levels of alkaloid-free coca leaf extract (AFCE). 96 15-wk-old quail hens, individually caged, were used in this study. Sixteen quail hens received, for 10 weeks, one of the following treatments: T1, Basal diet (BD, Corn and SBM only); T2, BD + 0.05% commercial pigment;; T3, BD + 0.01% AFCE, T4, BD + 0.03% AFCE, T5, BD + 0.05% AFCE; T6, BD + 0.07% AFCE. Feed (as mash) and fresh water were provided ad libitum. External (specific gravity, weight, shell weight and shell thickness) and internal (yolk weight, yolk pigmentation and Haugh Units) characteristics of eggs were measured in 360 individual eggs (60 eggs per treatment), collected at 4, 6, 8 and 10 wk of the experimental period. Statistical significance was evaluated using ANOVA under a Randomized Complete Block Design with Tukey's test for multiple comparisons. The results showed that, with the exception of yolk pigmentation, the other external and internal characteristics determined were not significantly influenced ($P > 0.05$) by diet treatment. Eggs from birds that were fed T2 (diet with a commercial pigment) showed the highest ($P < 0.05$) yolk pigmentation as measured using the Roche yolk color fan. Birds that were fed diet (T6) produced eggs that were more pigmented ($P < 0.05$) than those that were fed the basal diet (T1). It conclusion, the alkaloid-free coca leaf extract can be used in quail diets as a potential novel pigment source, especially when the price or the availability of commercial pigments are compromised.

Key Words: quail hen, egg quality, shell thickness, yolk color, coca leaf extract

330 The effects of CreAMINO supplementation to broiler breeder hens on creatine levels in the hatching egg and expression of creatine transporter and synthesis genes in their progeny. N. Reicher*[1], T. Epstein[1], D. Gravits[1], M. Rademacher-Heilshorn[2], and Z. Uni[1], *[1]Hebrew University, Rehovot, Israel, [2]Evonik Industries, Hanau-Wolfgang, Germany.*

Supplementation of broiler breeder hens with beneficial additives affects nutrient deposition in the hatching egg. GAA (guanidinoacetic acid), a precursor of creatine, plays a key role in cellular energy metabolism and is therefore used as a feed additive in poultry. The current study aimed to determine the effects of broiler breeder supplementation with 0.15% CreAMINO (GAA supplement) on creatine absorption and transfer from hen to egg and the subsequent effects on embryonic creatine absorption and synthesis. In this study, 16 47-week-old Cobb500 broiler breeder hens were kept in individual cages until 64 weeks. The hens were randomly divided into 2 groups: a control group with no CreAMINO in their diet (Control) and a treatment group which was supplemented with 0.15% CreAMINO in their diet. Eggs at day of lay were collected at 58 weeks (following 11 weeks of CreAMINO supplementation) for determining yolk and albumen creatine concentrations. Hens and progeny from Control and CreAMINO groups were sampled at 64 weeks (following 17 weeks of CreAMINO supplementation) for qPCR analysis: the creatine transporter gene (CRT) was examined in hens' small intestines and oviducts, as well as in the small intestines of their progeny during the last days of incubation: E17, E19 and DOH. Furthermore, the AGAT and GAMT genes, which encode enzymes involved in creatine synthesis, where examined in the kidneys and livers of progeny at DOH. Results showed a 21% increase in albumen creatine and a 52% increase in yolk creatine in hatching eggs of the CreAMINO group, compared with the Control group. The CreAMINO group exhibited significantly increased CRT expression ($P \leq 0.05$) compared with the Control group in their small intestines (1.5-fold) and oviducts (2.25-fold). These results demonstrate an increased ability of creatine absorption and transfer from broiler breeder feed, through the small intestine and oviduct, to the hatching egg. As for progeny, a significant decrease by 1.4-fold in CRT expression at E19 and by 1.3-fold at DOH ($P \leq 0.05$) was found in the small intestines of the CreAMINO group progeny, compared with the Control group progeny. At DOH, creatine synthesis genes exhibited significant decreases in expression ($P \leq 0.05$) in theCreAMINO group progeny compared to the Control group progeny: kidney AGAT expression decreased 1.6-fold and liver GAMT expression decreased 1.9-fold. Since cellular arginine and glycine are required for creatine synthesis, this downregulation of creatine synthesis genes may result in increased availability of arginine and glycine for the benefit of the developing embryo.

Key Words: creatine, CreAMINO, broiler breeder, gene expression

331 The effect of three different oil sources on egg size, egg fatty acid profile, and egg yolk:albumen ratio for hatching eggs from broiler breeder parent stock. A. Beitia*[1], J. Caldas[2], K. Hilton[1], G. Mullenix[1], J. Weil[1], P. Maharjan[1], M. Schlumbohm[1], J. England[1], and C. Coon[1], *[1]University of Arkansas, Fayetteville, AR, [2]Cobb-Vantress, Siloam Spring, AR.*

Past studies showed that different oil sources can significantly alter egg yolk fatty acid profile (Rowghani et al., 2007; Skrtic et al., 2008). Corn and canola oils have a different fatty acid profile and previous research at University of Arkansas has shown benefits to embryonic development and early chick performance due to fatty acid supplementation (Boonsinchi et al., 2015). The objective of the study was to evaluate the effects of 3 different oil sources on egg size, the fatty acid profile of the egg, and egg yolk: albumen ratio of broiler breeder parent stock (PS). 1,008 pullets were reared in floor pens and later transferred to individual cages and light stimulated at 21 wks. From 1 to 21wk, pullets were control fed to meet target Cobb 500FF target body weight (BW) for parent stock pullets. All treatments were fed pullet starter, 0 to 28d; pullet grower I, 5 to 15wk; pullet grower II, 16 to 24 wk. At 24wk, a total of 162 pullets were randomly assigned to 3 different dietary treatments consisting of 54 hens per treatment. The first treatment, which represented a standard broiler breeder diet, served as a control and contained poultry fat as the oil source. The second treatment contained corn oil as the oil source, while the third treatment contained canola oil. Treatment 1, 2, and 3 were isocaloric diets with 2925 kcal/kg and a fat addition of 2.10%. Egg size, the fatty acid composition of the egg, and egg composition was determined at 29, 39, and 50 weeks of age. Egg composition was measured by dual-energy x-ray absorptiometry (DEXA) to determine yolk: albumen ratios. Data was analyzed using JMP Pro 13 (SAS, 2016). Egg size significantly increased from hens fed corn oil ($P < 0.05$) in comparison to canola oil and poultry fat at 29 and 39 weeks; however, there was no significant effect on egg size for wk 50 ($P = 0.8179$). The amount of stearate and linoleate acid (C18:0 and C18:2, respectively) was significantly higher in eggs from hens fed corn oil ($P < 0.05$) in comparison to canola oil and poultry fat at 29, 39, and 50 weeks of age. Eggs from hens fed poultry fat had a higher yolk: albumen ratio ($P < 0.05$) in comparison to eggs from hens fed corn and canola oil at 29, 39, and 50 weeks. Results from this study showed corn oil supplementation improved early egg size and amount of C18:0 and C18:2 in eggs from broiler breeder parent stock.

Key Words: fatty acid composition, egg composition, egg size, oil sources, broiler breeder

332 Effect of different additives on laying hen performance and egg quality parameters. C. Sol*[1], M. Puyalto[1], A. Bertechini[2], V. Cantarelli[2], and J. J. Mallo[1], *[1]NOREL S.A., Madrid, Madrid, Spain, [2]Universidad Federal de Lavras, Lavras, Brazil.*

This study was performed to evaluate the effect of different additives on performance and egg quality parameters in laying hens. A total of 600 laying hens Hy-line W36 with 56 weeks of age were distributed in 5 treatments (n = 10): T1 - Control feed without additives, T2 -QUALITEGG (1.5Kg/tn of feed), based on organic acids (sodium butyrate, propionate salts and formate salts), organic trace minerals (methal methionine), and vitamins; T3 - EGG'XEL (1Kg/tn), based on sodium butyrate, and organic trace minerals (methal methionine); T4 - GUSTOR N'RGY (0.5Kg/tn), sodium butyrate protected with sodium salts of palm fatty acids distillates; T5 - GUSTOR BCP5 (0.5Kg/tn), calcium butyrate protected with vegetable fat. The study lasted 9 weeks, from wk 56 to 65. The parameters evaluated were: feed intake (FI), eggs production (EP), egg mass (EM), feed conversion ratio (FCR) and FCR per dozen (FCR/dz), egg weight (EW), broken eggs (BE), eggshell thickness (EST), specific weight (SW), eggshell percentage (ESP), yolk color (YC), Haugh Units (HU), albumen % (A%) and yolk % (Y%). The results were analyzed by one way ANOVA using the GLM procedure of SAS. There were no differences in FI. For EP (%), T4 and T5 had statistically higher EP than T2 and T3 and those higher than T1 (87.2c, 90.7b, 90.9b, 91.7a, 92.3a, for T1, T2, T3, T4 and T5, $P < 0.001$). For EM, T1 was significantly lower than T2, T3, T4 and T5 ($P < 0.001$). In FCR and FCR/dz, T1 was the highest and T5 the lowest (1.46d, 1.40c, 1.40bc, 1.39ab, 1.38a, for T1, T2, T3, T4 and T5, $P < 0.001$). There were no differences in EW. The percentage of BE was significantly higher for T1 (4.4a, 3.7ab, 3.5b, 2.9b, 3.3b, for T1, T2, T3, T4 and T5, $P = 0.05$). The EST, SW, ESP were significantly lower for T1 ($P < 0.001$). The internal quality only presented differences among treatments for HU, being significantly lower for T1 and higher for T5 (84.5c, 87.2b, 87.3b, 88.0ab, 88.5a, for T1, T2, T3, T4 and T5, $P < 0.001$). Those results indicated the there were significant effects of the additives on the performance and egg quality. It can be concluded that the better results in EP, FCR, FCR/dz and HU were achieved with T4 and T5, GUSTOR N'RGY and GUSTOR BCP5, respectively, due to both additives had high percentages of butyric acid in their formulation, equal or higher than 56%, respect to T2 and T3, QUALITEGG and EGG'XEL, with less than 15% of butyric acid. However, despite of QUALITEGG and EGG'XEL have less percentage of butyric acid, the right combination of organic trace minerals, and vitamins in their formulations, also exerted a reinforcement in the properties of the products that was demonstrated in the results.

Key Words: additives, sodium butyrate, calcium butyrate, egg quality

333 The effects of microencapsulated sodium butyrate supplementation on laying performance in Ross-308 broiler breeders fed antibiotic-free diets. J. Hou*[1], L. Zhe[1], R. Yu[1], S. Li[2], and Z. Li[2], *[1]Biological Feed Safety and Pollution Prevention and Control National Engineering Laboratory, Hangzhou, Zhejiang, China, [2]Provincial Key Agricultural Corporate Academy of Zhejiang, Hangzhou, Zhejiang, China.*

Butyrate, produced by bacterial fermentation, is a key energy source for intestinal epithelial cells. Research has shown positive impacts of butyrate on intestinal barrier integrity, feed efficiency, and immune function in poultry. The object of this study was to evaluate a 30% microencapsulated sodium butyrate with an intestine-specific delivery system (CMP; King Techina Group, China) on the laying performance and economic return in Ross-308 broiler breeders. A total of 120,000 43-week old Ross-308 broiler breeders (housed in 10 production buildings) were randomly assigned to 2 treatments consisting of 5 replicates (buildings) with 60,000 broiler breeder hens per treatment. The 2 treatments included: control group fed an antibiotic-free basal diet, and CMP group, supplemented with 500 mg/kg CMP to the basal diet. The experiment lasted for 9 weeks during which time laying performance was evaluated by egg production, hatchability, mortality, egg weight and economic returns. All laying performance indices were collected every 3 weeks. Data were analyzed by *t-test* via SPSS. Results showed that, in this experiment, CMP significantly increased the laying rate ($P < 0.05$) by 2.03%, decreased mortality ($P < 0.05$) by 1.55%, and had a trend toward enhancing egg mass ($P = 0.09$). There was no significant difference in hatchability ($P > 0.1$) between the 2 treatment groups. The economic analysis found that net profit from the CMP group improved by $0.31 per hen, and the return on investment ratio was 6.5 to 1. In conclusion, CMP supplementation can improve the laying performance, reduce the mortality rate, and increase economic returns without affecting egg weights or hatchability in Ross-308 broiler breeders.

Key Words: sodium butyrate, microencapsulation, broiler breeder, laying performance

334 Epigallocatechin-3-gallate protected vanadium-induced eggshell depigmentation via P38MAPK-Nrf2/HO-1 signaling pathway in laying hens. J. Wang*, Z. Yuan, K. Zhang, X. Ding, S. Bai, and Q. Zeng, *Sichuan Agricultural University, Chengdu, China.*

It has been demonstrated that tea polyphenol (TP) epigallocatechin-3-gallate (EGCG) can confer protection against vanadium (V) toxicity in laying hens; however, our understanding of the molecular mechanisms beyond this effect are still limited. In this study, 360 hens were randomly assigned to the 3 groups to study whether the potential mechanism P38MAPK-Nrf2/HO-1 signaling pathway is involved in the protective effect of EGCG on eggshell pigmentation in vanadium challenged laying hens. Treatments included a control group, a 10 mg/kg V (V10), and a V10 plus 130 mg/kg of EGCG group (V10+EGCG130). On study d 35, Twenty-four eggs per treatment (4 eggs/replicate) were collected to measure eggshell color and pigmentation content. Then, 72 hens (4 birds/replicate, 24 birds/treatment) were randomly chosen and sacrificed by cervical dislocation. Uterus (middle site) segments were immediately removed for morphology, apoptosis assay, and RT-PCR analysis. Eggshell color and protoporphyrin IX were decreased in the V10 group compared with the control diet, while EGCG130 treatment partially improved shell color and protoporphyrin IX ($P < 0.05$). The V10 exposure induced higher cell apoptosis rate and oxidative stress in birds as evidenced by the histological apoptosis status, decreased uterine glutathione-S transferase (GST) and high abundance of malondialdehyde (MDA) compared with the control group, whereas EGCG130 markedly alleviated oxidative stress via reducing MDA generation ($P < 0.05$). Dietary vanadium reduced ferrochelatase, NF-E2-related factor 2 (Nrf2), and heme oxygenase (HO-1) mRNA expression, while EGCG upregulated Nrf2 and HO-1 expression ($P < 0.05$). Protein levels of Nrf2, HO-1 and phospho-p38 (P-P38) MAPK were reduced in V10 group, while dietary supplementation with 130 mg/kg EGCG markedly increased Nrf2, HO-1 and P-P38 MAPK protein levels in the uterus compared with the V10 group ($P < 0.01$). In conclusion, EGCG improved eggshell color and antioxidant system in V10-challenged hens, which seems to be associated with P38MAPK-Nrf2/HO-1 signaling pathway.

Key Words: vanadium, epigallocatechin-3-gallate, eggshell color, P38 MAPK, Nrf2

335 Effect of organic acids as replacement of antibiotic growth promoter on growth performance, carcass characteristics and immune response in broilers. W. Yousaf, S. Bhatti, and T. Mahmood*, *University of Agriculture, Faisalabad, Faisalabad, Punjab, Pakistan.*

The study was planned to evaluate the effects of organic acid as substitute of antibiotic growth promoter on growth performance, carcass characteristics and immune response in broilers. For this purpose, 7-hundred-day old broiler chicks were randomly divided into 7 treatments having 4 replicates of 25 chicks each. Basal Starter (CP: 24%; ME: 3000 kcal/kg), grower (CP: 22%; ME 3100 kcal/kg) and finisher diets (CP: 20%; ME: 3200 kcal/kg) were formulated to act as control and were supplemented with Enramycin; Komiquindox (Olaquin); Maxus (Avial); Enradin (Enradin); organic acid blend (Silo-Health) or butyric acid C4 at rate of 0.0125%, 0.01%, 0.01%, 0.0075%, 0.01% or 0.1% respectively. Starter, grower and finisher diets were fed to birds from d 1 to 9, 10 to 20 and 21 to 35, respectively. Feed intake and body weight was measured weekly. At the end of trial, one bird from each replicate was randomly slaughtered for carcass parameters. Blood samples were collected (1 bird from each replicate) at 35th day for determining antibodies titers against New Castle Disease. Results revealed that feed intake, weight gain, FCR, European production index, protein intake, energy intake, protein and energy efficiency ratio during overall period were better ($P < 0.05$) in birds fed diet supplemented with organic acid or antibiotics than those fed control. However, these were not different ($P > 0.05$) in birds fed either organic acid or antibiotics. No differences ($P > 0.05$) of dietary treatments was observed in mortality, dressing percentage, breast meat, thigh meat and giblet organs (liver, heart and gizzard) of broilers. Immune response was not affected by dietary supplementation of organic acid and antibiotics. It is concluded that antibiotic growth promoters can safely be replaced with organic acids without affecting growth performance, carcass characteristic and immune response of broilers.

Key Words: antibiotic growth promoter, organic acid, growth performance, immune response, broiler

336 Economic benefits of using NutriOpt precision nutrition solutions in broiler diet formulation. G. Page[1], H. Romero-Sanchez[2], and J. Steed*[2], *[1]Trouw Nutrition Agresearch, Guelph, ON, Canada, [2]Trouw Nutrition USA, Highland, IL.*

With up to 70% of production costs associated with nutrition, it is increasingly important to critically review nutritional strategies to improve performance and economic returns. Trouw Nutrition has commercialized over 50 years of research under NutriOpt, a modular precision-feeding system designed to improve financial and animal performance objectives through advanced nutritional analysis and modeling. The aim of the present study was to evaluate the economic impacts of selected NutriOpt nutritional formulation solutions under typical US production conditions. 2208 Cobb 500 d of hatch cockerels were randomly allocated to one of 6 treatment groups, with 16 replicates of 23 birds/pen (2 rooms of 8 replicates). Treatments included: 1) control (consultant-formulated typical US diets), 2) shadow-formulated to 1 using NutriOpt NIR and raw material database (NIR), 3) as 2, using TN recommended AA profile (TNAA), 4) as 3, using NutriOpt rPhos and phytase dosing matrix (Phyt), 5) Optimized for margin per broiler using NutriOpt broiler model (Margin), and 6) Optimized for margin per bird for breast meat yield (BMY). The experiment was conducted under commercial conditions at the University of Georgia, using a 4 phase corn-soy-DDGS- meat meal based crumble/pelleted program: d0-d14, d14-d28, d28-d42, d42–49. Body weight (BW) and feed intake were assessed by pen on d 0, 14, 28, 42 and 49. Carcass yield parameters were assessed on 7 randomly selected birds in each pen on d 50. Data was analyzed in PROC GLIMMIX of SAS with treatment and period as fixed effects, and room/block treated as random effects. The observed growth performance exceeded commercial Cobb targets grown to 49 d with an overall average BW 4.12 kg. Mean treatment weights were not significantly different to the control ($P > 0.05$), although BMY trt was heavier (+2.8%; $P < 0.05$) than TNAA and Phyt trts. Cumulative FCR was significantly higher in Phyt trt vs. control (+2.0%), while BMY trt was significantly lower than control (−3.2%; $P < 0.05$). Treatments' total breast meat yields (pectoralis major + minor) were not significantly different from the control ($P > 0.05$), although the BMY trt produced 0.3% more breast meat. Relative to the control, intake-weighted feed costs ($/tonne) were significantly ($P < 0.05$) reduced by all treatments (by up to $8.54/tonne) except BMY trt. Margins (returns – costs; $/kg BW) were not affected by trt ($P > 0.05$), although were numerically equivalent or higher in all treatments except BMY, relative to control. The results suggest that NutriOpt precision nutrition formulation strategies can support equivalent performance with important reductions in feed costs.

Key Words: broiler, NIR, performance, NutriOpt, economics

337 Effect of the level and source of crude fiber on growth performance and organ weights of 21-day-old broilers. C. Uculmana*[1], I. Meza[2], and C. Vilchez[2], *[1]Laboratorios Veterinarios Gammavet SAC, Lima, Peru, [2]Universidad Nacional Agraria la Molina, Lima, Peru.*

The objective of this study was to determine the effect of the level and source of crude fiber (CF) on growth performance and organ weights of 21 d-old broilers. 200 one-day old Cobb 500 male chicks were distributed in 40 experimental units (EU) with 5 chick each one. Eight EU were fed, for 21 d, with one of the following treatments: T1, Control diet (corn and SBM only; 2.6% CF); T2, Diet with corn and SBM + ARBOCEL; 3.0% CF); T3, Diet with corn and SBM + wheat bran; 3.0% CF); T4, Diet with corn and SBM + ARBOCEL; 3.14% CF); T5, Diet with corn and SBM + wheat bran; 3.14% CF). All mash diets were isonitrogenous and isocaloric, and offered ad libitum along with fresh water. Live weight, weight gain, feed intake and feed conversion rate were recorded weekly. At d 21, all birds were sacrificed by cervical dislocation and their proventriculus, gizzard, liver, intestines, pancreas and ceca were weighed. Statistical significance was evaluated using ANOVA under a Completely Randomized Design with Tukey's test for multiple comparisons. The results showed that the live weight, weight gain and feed conversion rate were not affected by the dietary treatments ($P > 0.05$), but low feed intakes were observed ($P < 0.05$) in the groups of birds that received diets that contained ARBOCEL (T2 and T4) without affecting the growth performance. In comparison to the T1, birds that were under T5 had higher gizzard weight (+26.9%; $P < 0.05$), higher intestines weight (+20.1%; $P < 0.05$) and lower carcass weight (−1.43%, $P < 0.05$); however, the birds that received diets that contained ARBOCEL (T2 and T4) did not show differences ($P > 0.05$) in above mentioned variables when compared with those of the control group (T1). In conclusion, the growth performance tends to be slightly affected by the increase of the dietary crude fiber level using wheat bran as a source fiber rather than an insoluble fiber source such as ARBOCEL.

Key Words: insoluble fiber, ARBOCEL, crude fiber, wheat bran, broiler

338 Effect of dietary incorporation of processed bambara groundnut (*Vigna subterranea)* on broiler performance and carcass characteristics. M. Mahmoud* and A. Mohamad, *University of Gezira- Faculty of Animal Production, Wad Medani, Gezira, Sudan.*

The objective of the study was to investigate the effect of dietary incorporation of differently processed bambara nut with roasting (A) and boiling (B) on broiler performance. The processed bambara nut were ground and analyzed to be included in the experimental diets. The inclusion levels (treatments) of roasted bambara nut were 10% (A1), 15% (A2) and 20% (A3) and for boiled one were 10% (B1), 15% (B2) and 20% (B3). The control diet contained 0% bambara nut (C). The experimental diets were formulated iso-energetic and iso-nitrogenous to meet or exceed the requirements of broilers according to (NRC, 1994). A total of 210 one day-old Hubbard broilers were allocated to the 7 treatments (A1, A2, A3, B1, B2, B3 and C) and each treatment was further divided randomly into 3 replicates with 10 birds each. Feed consumption, weight gain and feed conversion ratio (FCR) were recorded. At the end of the experiment, 2 birds from each experimental unit were selected according to their closed to average weight of the experimental unit and slaughtered. Carcass weights, relative weights of organs and cuts were measured. Blood samples were collected for hematological profile and constituents. All data were statistically analyzed using ANOVA. The results showed that bambara nut tannin and phytate content were reduced by roasting and boiling treatments. Feed consumption, weight gain and FCR were not affected by experimental treatments. The weights of live body and carcass of birds fed on diets containing Bambara nut were greater ($P \leq 0.01$) than those birds fed on control diet. Numerically, the last body weights increased when the level of (A) increased. The relative weights of internal organs (proventriculus, gizzard, pancreas, spleen and abdominal fat) were not influenced by experimental treatments. Moreover, differential leucocytes counts were not influenced by experimental treatments. The greatest values of blood protein and cholesterol ($P \leq 0.01$) were observed with fed on A1

and B3 diets, respectively. The results indicate that, processed Bambara nut could be used as a source of protein in broiler diets to increase the weights of body and carcass without any adverse effects.

Key Words: Bambara groundnut, anti-nutritional factors, roasting, boiling, broiler

339 Effect of dietary protein source and litter condition on growth performance and meat yield of broiler chickens reared to 46 days of age. J. Starkey*[1], R. Shirley[2], A. Welsher[2], O. Tejeda[1], L. Spencer[1], D. Bourassa[1], and C. Starkey[1], *[1]Auburn University, Auburn, AL, [2]Adisseo USA Inc., Alpharetta, GA.*

The objective of this 3 × 2 factorial study was to determine whether growth and meat yield of broilers that were hatched and reared without antibiotics differed when: 1) fed different dietary protein sources, and 2) reared under different litter conditions. To investigate this, 3 dietary treatments were formulated to included either: 1) soybean meal (SBM), 2) poultry by-product meal + feather meal (50:50 blend; PBM), or 3) mammalian meat and bone meal (MBM). All diets were formulated to meet or exceed the nutrient recommendations of the primary breeder and were both iso-caloric and iso-nitrogenous. To define the effect of litter condition upon broiler performance, floor pens were bedded with either: 1) new, pine wood shavings (NEW) or 2) built-up litter from a prior broiler grow-out, top-dressed with new, wood shavings (USED). Upon arrival, day-of-hatch, male Yield Plus × Ross 708 broiler chicks (n = 1,800) were randomly assigned to 1 of 6 treatments each represented with 12 replicate pens (25 birds per 2.3 m^2 pen). Within each litter treatment, broilers received 1 of 3 diets in 4 phases: starter (d 1 to 12), grower (d 13 to 27), finisher (d 28 to 38), and withdrawal (d 39 to 46). Mortality-adjusted BW, feed intake (FI), and FCR were determined for each phase. On d 47, following a 24-h static water chill, broiler carcasses were deboned and part yields were determined. Data were analyzed using the MIXED procedure of SAS with pen as the experimental unit and block as a random factor. Means were considered significantly different when $P \leq 0.05$. No significant interactions between the main effects of protein source and litter condition were observed ($P \geq 0.1510$). On d 12, 27, 38, and 46, birds reared on NEW litter had significantly lower BW compared with those reared on USED litter ($P \leq 0.0189$). Birds reared on USED litter had greater FI during the starter, finisher, and withdrawal phases compared with those on NEW litter ($P \leq 0.0331$). During the starter, grower, finisher, and withdrawal phases, FCR was lower for broilers reared on USED litter ($P \leq 0.0238$). No significant differences in carcass or carcass part weights were observed among broilers reared on NEW and USED litter ($P \geq 0.3029$). While d 0 to 46 broilers fed PBM tended to have lower BW gain compared with those fed MBM or SBM ($P = 0.053$), FCR was similar among protein sources ($P = 0.437$). Broilers fed MBM and SBM yielded heavier carcasses ($P = 0.0149$) with less abdominal fat ($P = 0.0015$), and had heavier breast fillets ($P = 0.005$) and tenders ($P < 0.0001$) compared with those fed PBM. Feeding either MBM or SBM resulted in improvements in growth performance and meat yield compared with PBM, and USED litter conditions improved growth performance.

Key Words: broiler chicken, protein source, litter condition, growth performance, carcass yield

340 Different nutritional proposals on laying hen diets on performance, egg quality and body composition in post-peak egg production. F. Fabbri[2], M. Melare[2], M. Reis[2], N. Sakomura[2], A. Rech*[1], and L. Bittencourt[1], *[1]DSM Nutritional Products, Sao Paulo, Brazil, [2]UNESP, Jaboticabal, Brazil.*

Commercial egg producers use different nutritional supplements for feeding laying hens with different vitamin and mineral recommendations and sources as well as a wide variety of nutritional additives to improve production. Therefore, the objective was to evaluate the influence of different premix concepts on egg production of hens in post-peak. One hundred 60 63-weeks-old-HyLine W36 laying hens were randomly allocated to 2 treatments, 10 replicates of 8 birds each. All hens were fed a corn-SBM mash diets, to attend the breeder requirements. Treatments were: (T1) basal diet (BD) supplemented with a commercial premix (0,4%) vitamin levels and minerals (sulfate) an average of Latin America producers, enzymatic blend (phytase and carbohydrase) and zinc bacitracin; (T2) BD with concept premix (0,4%), with optimal vitamin nutrition (OVN), 25(OH)D_3, carbo-amino-phospho-chelate trace minerals, monocomponent enzyme blends (phytase, carbohydrase, amylase and protease), probiotic, organic acids, essential oils and pigments. Vitamins and minerals levels T1/T2, kg per feed: Vit. A 8,000/12,000 IU; Vit. D_3 2,000/3,000 IU; 25 (OH) D_3, 0/69 µg; Vit. E 10/30 mg; Vit. K_3 1,600/3,000 mg; Vit. B_1 1,000/3,000 mg; Vit. B_2 3,000/7,000 mg; Vit. B_6 2,000/5,000 mg; Vit. B_{12}, 10/25 µg; niacinamide, 18/50 mg; pantothenic acid 8/12 mg; folate 400/1,500 µg; biotin 60/150 µg; choline 260/500 mg; cooper 8/8.62 g; iron 50/43.74 g; manganese 65/56.37 g; zinc 60/43.74 g; selenium 300/340 mg. Experiment trial was 4 periods of 28 d each one, and were measured in the end of each period, productivity (feed intake, FI, egg weight, EW, lay, egg mass, EM, and feed conversion ratio, FCR), egg quality (Haugh Unit, HU, yolk color, YC, eggshell strength, ST, and thickness, ST) and body composition (fat mass, lean, bone mineral density and content, ash, fat, protein and water). Data were submitted to ANOVA and means compared by Tukey test at 5% probability. Effect of diet premix supplementation (commercial vs concept) was found ($P < 0.05$) for FI (102 vs 106, g); EW (67 vs 66, g), lay (84 vs 86, %) and FCR (1.65 vs 1.71, g:g). It also influenced the egg quality ($P < 0.05$) for YC (6 vs 11, Yolk Color Fan) and HU (85 vs 86), and body composition ($P < 0.05$), for fat mass (299 vs 253, g), protein (17.63 vs 18.03, %) and water (62 vs 63, %). The concept premix increased the laying with decreases in EW as expected, and improved internal egg quality. The improvements in productivity and quality in some way affect hen's body composition, probably due to the increases on reserves mobilization in post-peak. Based on results, it was concluded that the concepts premix affected positively the hen productivity, egg quality and body composition.

Key Words: vitamins, minerals, eubiotics, enzymes, hens

341 Supplemental microlagal docosahexaenoic acid enriched the fatty acid and affected expression of genes related to lipid metabolism in tissues of broiler chickens. S. Tolba[1], T. Sun[1], A. Magnuson[1], G. Liu*[1], W. Abdel-Razik[3], M. El-Gamal[2], and X. G. Lei[1], *[1]Cornell University, Ithaca, NY, [2]Zagazig University, Zagazig, Sharkia, Egypt.*

This experiment was to study how supplemental dietary microalgal docosahexaenoic acid (DHA) affected enrichments of this n-3 fatty acid and gene expression related to lipid metabolism in tissues of broiler chickens (BR). A total of 192 (day old) Cornish male BR were allotted to 4 dietary treatments (6 cages/treatment and 8 birds/cage). The birds were fed corn-soybean meal basal diet supplemented with a DHA- rich microalgal (*Aurantiochytrium*) biomass (Heliae, Gilbert, AZ) at 0, 1, 2 and 4% (0, 1.7, 3.4 and 6.8 g DHA/kg diet) for 6 weeks. Blood, liver, adipose and breast and thigh muscle samples (n = 6/treatment) were collected at wk 3 and 6 for analyses. Data were analyzed by one-way ANOVA and linear regression. The biomass inclusion resulted in dose- dependent ($P < 0.01$) enrichment of DHA in plasma ($R^2 = 0.76–0.79$), liver (R^2

= 0.70–0.75), breast (R^2 = 0.89–0.91), and thigh (R^2 = 0.91–0.93) at both wk 3 and 6. The highest DHA concentrations reached 0.82 and 0.96 g/kg in breast and thigh muscles, respectively, in chicks fed 4% of the biomass at wk 6. The biomass inclusion resulted in dose-dependent decrease ($P < 0.01$) of n-6 fatty acid concentrations (19–92%) and the n-6/n-3 fatty acid ratios (61–95%) in all assayed tissues, but elevated ($P < 0.01$) total polyunsaturated fatty acids concentrations by 66–91% in the liver at wk 3 and 6. Compared with the control, the 4% biomass decreased ($P < 0.01$) nonesterified fatty acid concentrations in liver and adipose tissue by 34 and 25%, respectively. Compared with the control, the DHA-rich microalgae supplementation decreased ($P < 0.05$) hepatic mRNA abundances of fatty acid desaturases1 (63–64%), fatty acid elongase2 (60–75%), fatty acid synthase (FAS) (45–49%), stearoyl-CoA desaturase1 (SCD1) (61–74%), sterol regulatory element binding protein1 (46–60%), carnitine palmitoyltransferase2 (17–44%), acyl-CoA synthetase (ACS) (53%), and interleukins6 (53%) and 10 (45%) at wk 3 and (or) 6. In contrast, the supplementation enhanced ($P < 0.05$) adipose tissue mRNA abundances of acetyl-CoA carboxylase (38–46%), FAS (33%), SCD1 (up to 2.6-fold), acyl-CoA oxidase1 (47%), acetyl-CoA acyltransferase2 (30–31%) and ACS (25–106%) at the 2 time points. In conclusion, supplementing the DHA-rich microalgae effectively enriched DHA and lowered n-6/n-3 fatty acid ratios in all assayed tissues of broiler chickens. However, the supplementation exerted differential impacts on expression of genes related to lipid metabolism between the liver and adipose tissue. Supported in part by Heliae, Glbert, AZ; DOE (DE-EE007091); and Cornell University (Hatch grant NYC-127419).

Key Words: broiler, DHA, enrichment, fatty acid, gene

342 Replacement of solvent-extracted soybean meal with untoasted full-fat soybean: Effects on growth performance, immune organs and blood chemistry in finishing broiler chickens. M. Zahid, M. Mirza, and T. Mahmood*, *University of Agriculture, Faisalabad, Faisalabad, Punjab, Pakistan.*

This study was planned to investigate if untoasted full-fat soybean (UFFSB) had any effect on the growth performance of finishing broilers chickens (d 21–35) when it replaced standard solvent extracted soybean meal (SBM) on protein-equivalent basis. Five iso-nitrogenous and iso-caloric (CP:21%; ME:3150 kcal/kg) diets were formulated by replacing SBM with UFFSB @ 0, 25, 50, 75 and 100% and designated as FFSB0, FFSB25, FFSB50, FFSB75 and FFSB100. One hundred and 50 (n = 150) one-day-old broiler chicks were randomly distributed under 5 dietary treatments. Each dietary treatment had 3 replicates with 10 broiler chicks/replicate. The diets were fed ad libitum for the experimental period (d 21–35). Data on feed intake and body weight of each replicate were recorded on weekly basis to calculate body weight gain and feed:gain. Feed intake by the birds during d 21–28 or 28–35 or 21–35 (overall-finishing stage) was not influenced by the dietary treatments. Body weight gain and feed:gain during d 21–28 were unaffected irrespective of the level of replacement of SBM with UFFSB. The body weight gain and feed:gain during d 28–35 and during the entire finishing stage (d 21–35) were observed to be impaired ($P < 0.001$) when SBM was replaced with UFFSB. Maximum drop in body weight gain (d 21–35) was observed in broiler chicks fed the FFSB100 diet followed by the FFSB75, FFSB50, FFSB25 and FFSB0 diets. Similar trend was observed in feed:gain. On d 35, one bird from each replicate selected on random basis was slaughtered to obtain data on soft organ weights. The weight of heart, liver, kidneys and immune organs (bursa, thymus and spleen), however, remained un-affected ($P > 0.05$) across the dietary treatments. Replacement of SBM with UFFSB had non-significant ($P > 0.05$) effect on hematological parameters. In conclusion, UFFSB cannot completely replace SBM in broiler chicken diets during finishing phase.

Key Words: full-fat soybean, broiler chicken, immune organ, blood chemistry, growth

343 Impact of mycotoxins, rancidity, particle size, wheat, and high-calcium levels on chicken live performance and digestibility. E. Oviedo-Rondón, V. San Martin, M. A. Wisaquillo*, C. Florez, A. Sarsour, H. Cordova-Noboa, J. Cifuentes, I. Martinez, and S. Alvarez, *North Carolina State University, Raleigh, NC.*

Feed composition, particle size and quality of ingredients have been reported to impact nutrient digestibility and live performance. However, limited studies have compared the effects of these factors under similar conditions to determine which one has more relevance. Therefore, the objective of this study was to evaluate the impact of naturally occurring mycotoxins in moldy corn, oil rancidity, particle size of corn, inclusion of wheat and high-calcium levels on live performance, digestibility and organ development in chickens. A total of 336 d-old Ross-708 female chicks were randomly distributed in 48 pens. Eight dietary treatments were randomly distributed to have 6 replicate pens of 7 chicks each. Diets were formulated to have similar nutritional content and included: corn coarse (900 μm) and fine (400 μm) grinding, high-calcium (1.40%) with poultry fat, high Ca with soybean oil, standard Ca (1.10%) with soybean oil, mycotoxins (moldy corn stored for 2 years and naturally contaminated with aflatoxin B_1 142 ppm, aflatoxin B_2 19.5 ppm, aflatoxin G_1 3.1 ppm, fumonisin B_1 2.2 ppm, fumonisin B_2 0.7 ppm, and fumonisin B_3 0.2 ppm), rancid soybean oil (heated 65°C for 14 d with peroxide value of 16 mEq/kg) and wheat replacing all corn. Each diet was fed from 1 to 14 d of age and chickens had similar management and environmental conditions with mild heat stress (28–35°C) during the second wk. BW and feed intake were obtained, BW gain, FCR, European Production Efficiency Factor (EPEF) and flock uniformity calculated. Data were analyzed in a completely randomized design with diet characteristics as treatments and pen location within room as random effect using a one-way ANOVA with orthogonal contrasts. Greater BW and BW gain were found in chickens fed diets with corn coarse grinding and wheat-based diets, in contrast diets with mycotoxins had the lightest chickens. The best FCR ($P < 0.05$) was observed in chickens fed high-Ca-poultry fat diets, but similar to chickens fed diets with corn coarse grinding and high-Ca-soybean oil. On the other hand, diets with rancid oil and wheat inclusion had an intermediate FCR in comparison to chickens fed diets with mycotoxins which had the worst FCR. There were no effects ($P > 0.05$) of treatments on flock uniformity. The EPEF was similar for all treatments except for the diet with mycotoxins that had an inferior index. Dry matter and crude protein digestibility results suggested that mycotoxins in feed decreased ($P < 0.05$) digestibility as compared with other treatments. No signs of intestinal health problems were observed. In conclusion, from all treatments, the diet containing moldy corn with mycotoxins reduced digestibility and consequently impaired live performance up to 14d.

Key Words: live performance, mycotoxin, wheat, corn, protein digestibility

344 Growth performance and carcass characteristics of broilers raised under different feeding programs that contained hominy grits. W. Castañeda and C. Vilchez*, *Universidad Nacional Agraria la Molina, Lima, Peru.*

An experiment was carried out to determine de effects of different feeding programs that contained hominy grits (HG) on growth performance and carcass characteristics of broilers. 720 one-day-old Cobb 500 male chicks were used. From d 1 to d 8 all birds were fed a common pre starter diet and then a 3-phase feeding program (FP): starter (S; 9 to 21 d), grower (G; 22 to 35 d) and finisher (F; 36 to 42 d). The levels of inclusion of HG in each FP were as follow: FP1, Control group; No HG in any phase; FP2, 5% HG in S, G and F diets; FP3, 5%, 10% and 15% HG in S, G and F diet, respectively; FP4, 10%, 15% and 20% HG in S, G and F diet, respectively. Each FP consisted of 6 replications with 30 birds per replication. Feed (as mash) and fresh water were offered ad libitum. Average body weight, body weight gain, feed intake, feed conversion ratio and mortality (%) were determined weekly. On d 43, 3 birds were randomly selected from each replicate (18 birds/treatment) to evaluate for carcass characteristics (carcass yield, breast yield and abdominal fat; %). Statistical significance was evaluated using ANOVA under a Complete Randomized Design with Tukey's test for multiple comparisons. The results showed that neither growth performance nor carcass characteristics were significantly influenced ($P > 0.05$) by any of the feeding programs considered in the present study; however, when the cost per kilogram of bird produced is taken into account, the lowest cost corresponded to the FP4 feeding program. In conclusion, hominy grits can be used in broiler feeding without affecting live performance or carcass characteristic with a greater gross margin per bird when compared with a feeding program without it.

Key Words: hominy grits, broiler, carcass characteristic, feeding program, performance

345P Effects of Original XPC or AviCare on stress susceptibility, plasma chemistry, cytokine levels, and antioxidant status of broiler chickens. J. Nelson*, E. Sobotik, and G. Archer, *Texas A&M University, College Station, TX.*

Previous research has shown the ability of Original XPC to reduce stress and improve growth in a variety of livestock. This study investigated the effects of XPC and its liquid counterpart, AviCare, on stress susceptibility, electrolyte status and plasma chemistry, antioxidative capability, and cytokine levels in broiler chickens exposed to acute stress and normal rearing stressors. Day-old male broiler chicks were assigned to one of 3 treatments: stressed control (CS), stressed and supplemented with XPC (1.25kg/MT feed; XPC) d0–42, or stressed and supplemented with AviCare (160mL/100L drinking water; AVI) d0–42. All birds were vaccinated for coccidiosis on d0, raised on reused litter, vaccinated for Newcastle/Bronchitis on d18, and exposed to heat stress with feed/water withdrawal for 12h on d18. Plasma corticosterone (CORT) and heterophil/lymphocyte ratio (H/L) were determined in 60 birds/T on d40. Plasma chemistry with electrolytes and growth hormone were evaluated in 12 birds/T on d38. Serum serotonin and plasma levels of prolactin, T3, T4, creatine kinase, superoxide dismutase, FRAP, and cytokines were evaluated in 12 birds/T on d39. Physical asymmetry of bilateral bone traits (ASYM) was obtained from 60 birds/T on d41. Data was analyzed using GLM: a significant difference was considered $P < 0.05$. Results showed that plasma phosphorus was lower in AVI than CS ($P = 0.021$); alkaline phosphatase activity (ALP) was lower in AVI than XPC ($P = 0.027$); and serotonin was lower in CS than XPC ($P = 0.049$). IL-1β was higher in AVI birds compared with both CS ($P = 0.009$) and XPC ($P = 0.009$). CS birds had higher CORT than both AVI ($P = 0.013$) and XPC ($P = 0.037$), as well as higher H/L ratios than AVI ($P = 0.026$) and XPC ($P = 0.034$) birds. There were no other differences among treatments. Both AviCare and XPC improved welfare of broilers by reducing stress susceptibility during normal rearing stress. However, XPC and AviCare produced different effects on protein metabolism and pro-inflammatory cytokine production. As such, XPC may be slightly more effective than AviCare at mitigating physiological effects associated with the stress response in broilers exposed to normal rearing stressors.

Key Words: stress, broiler, XPC, AviCare

346P Evaluating the ability of AviCare^{T} administered at different concentrations in the water to reduce stress in broilers chickens. E. Sobotik*[1], J. Nelson[1], D. McIntyre[2], H. Pavilidis[2], and G. Archer[1], *[1]Texas A&M University, College Station, TX, [2]Diamond V, Cedar Rapids, IA.*

Supplementation of poultry diets with AviCare has been proposed as a means to ameliorate production challenges, especially during periods of acute heat stress. A study was conducted to evaluate the effects of administering AviCare\to broilers reared under heat stress temperatures. Dietary treatments included: 1) Control that was stressed and untreated (CON); 2) AviCare (AviLow) - birds stressed while consuming AviCare (80 mL/100L) in the water; 3) AviCare (AviMid) - birds stressed while consuming AviCare (120 mL/100L) in the water; 4) AviCare (AviHigh) - birds stressed while consuming AviCare (160 mL/100L) in the water; 5) Original XPC (XPC) (1.25 kg/MT), in the feed. On Day 18, feed and water was removed from all pens, pen space was reduced, and litter temperature was increased (32°C-35°C) for a period of 12 h. On d19 and d41 blood was collected from 5 birds/pen. Blood was analyzed for plasma corticosterone concentrations (CORT) and heterophil/lymphocyte rations (H/L). Data was analyzed using GLM: a significant difference was considered $P < 0.05$. On d19, CON had higher CORT than AviHigh and XPC ($P < 0.05$; 3806.3 ± 655.3 vs. 2055.7 ± 323.3 (AviHigh); 2197.4 ± 313.7 (XPC). On d42, CON had higher CORT values than AviHigh and XPC ($P < 0.05$; 1776.7 ± 177.9 vs. 1251.2 ± 166.6 (AviHigh); 1192.2 ± 185.3 (XPC)). CON had a higher H/L ratio that all other treatments on d19 ($P < 0.05$). On d42, CON had higher H/L ratio than AviHigh and XPC ($P < 0.05$; 0.68 ± 0.06 vs. 0.50 ± 0.05 (AviHigh); 0.51 ± 0.04 (CON)). Physical asymmetry scores were also higher in CON compared with all other treatments ($P < 0.05$; 1.44 ± 0.15 vs. 0.98 ± 0.08 (AviLow); 1.13 ± 0.12 (AviMid); 1.13 ± 0.08 (AviHigh); 1.05 ± 0.08 (XPC)). Feeding XPC or administering AviHigh in the water improved the welfare of broilers while under acute or normal rearing stressors. Results from this study indicate that supplementing AviCare may reduce the negative attributes associated with heat stress, ultimately improving poultry welfare.

Key Words: stress, broiler, XPC, AviCare

347P The effect of raising Pekin ducks two spectra of LED light on production, stress, and behavior. G. House*, E. Sobotik, J. Nelson, and G. Archer, *Texas A&M University, College Station, TX.*

Light-emitting diode (LED) light bulbs are becoming more prevalent in poultry production but their using in duck production in the USA is limited. While there is some research on how spectrum of light can affect ducks, little has been conducted on how it may affect stress and fear. To determine how different spectrum of light produced by LED lights could affect production, stress, and behavior we raised ducks under either a white LED with a large amount of blue light (BLUE) or a large amount of red light (RED) in 2 trials. To determine stress susceptibility bilateral asymmetry (ASYM, n = 120), humoral immunity (KLH, n = 40), heterophil to lymphocyte ratios (HL, n = 40), plasma IL-12 concentrations (IL12, n = 40) and plasma corticosterone concentrations (CORT, n = 40) were measured. Fear was measured using tonic immobility (TI, n = 120) and inversion (INV, n = 120). Weight gain and feed conversion (FCR) were also determined. Data was analyzed using GLM: a significant difference was considered $P < 0.05$. The RED birds had lower CORT (449.8 ± 53.4 ng/dl, $P = 0.04$) and HL (0.40 ± 0.03, $P = 0.03$) than the BLUE birds (651.8 ± 79.5 ng/dl and 0.58 ± 0.08). The RED birds righted faster during TI (25.7 ± 2.3 s, $P < 0.001$) compared with BLUE birds (58.8 ± 8.9 s). There was no difference observed between treatments in ASYM, KLH, IL12, or INV ($P > 0.05$). There was also no difference between treatments in final body weight or feed conversion ($P > 0.05$). These results demonstrate that raising ducks under different spectrums of light can affect their stress and fear response, though it had no effect on growth or feed conversion. These results once again indicate that it is important to use the correct lighting to optimize bird welfare.

Key Words: duck, light, spectrum, stress, fear

348P The effect of utilizing ultraviolet light while rearing broiler chickens: Production, stress, and fear. J. Nelson*, E. Sobotik, and G. Archer, *Texas A&M University, College Station, TX.*

Artificial lighting used in poultry production lacks the UV part of the light spectrum. Poultry can see into the UV spectrum unlike humans and little research has been done to investigate the importance of this

part of the spectrum on bird welfare. To determine if adding UV light to typical LED lighting could affect production, stress, and behavior we raised broilers under either just a commonly used LED fixture (Control) or with the addition of UV light (UV). Two trials were conducted as study replication. To determine stress susceptibility bilateral asymmetry (ASYM, n = 120), humoral immunity (KLH, n = 40), heterophil to lymphocyte ratios (HL, n = 24), and plasma corticosterone concentrations (CORT, n = 24) were measured. Fear was measured using tonic immobility (TI, n = 120) and inversion (INV, n = 120). Weight gain and feed conversion (FCR) were also determined. Data was analyzed using GLM: a significant difference was considered $P < 0.05$. The UV birds had lower CORT (66.5 ± 9.6 ng/dl, $P = 0.04$), ASYM (1.25 ± 0.06, $P < 0.001$) and HL (0.32 ± 0.03, $P = 0.03$) than the Control birds (230.3 ± 55.1 ng/dl, 1.75 ± 0.11 and 0.60 ± 0.06). The UV birds righted faster during TI (129 ± 2.3 s, $P < 0.001$) and flapped less intensely during INV (4.73 ± 0.28 flaps/sec, $P < 0.001$) compared with Control birds (293 ± 8.9 s and 7.13 ± 0.20 flaps/sec). There was no difference between treatments in final body weight or feed conversion ($P > 0.05$). These results demonstrate that rearing broilers with UV light as part of the lighting spectrum can reduce their stress and fear response, though it had no effect on growth or feed conversion. These results once again indicate that it is important to use the correct lighting to optimize bird welfare and that having more natural lighting can help maximize that welfare.

Key Words: broiler, light, fear, stress, ultraviolet

349P The effect of utilizing ultraviolet light while rearing laying hens: Production, stress, and fear. E. Sobotik*, J. Nelson, and G. Archer, *Texas A&M University, College Station, TX.*

Artificial lighting used in poultry production lacks the UV part of the light spectrum; however, poultry can perceive the UV spectrum unlike humans and little research has been done to investigate the importance of this part of the spectrum on bird welfare. To determine if adding UV light to typical LED lighting could affect production, stress, and behavior we reared laying hens from 18 to 72 weeks of age under either just a commonly used LED fixture (Control) or with the addition of UV light (UV). Three replicate rooms with each lighting type sharing an air space were used in this study. To determine stress susceptibility bilateral asymmetry (ASYM, n = 120), humoral immunity (KLH, n = 40), heterophil to lymphocyte ratios (HL, n = 24), and plasma corticosterone concentrations (CORT, n = 24) were measured at 3 time points during production. Fear was measured using tonic immobility (TI, n = 120) and inversion (INV, n = 120) also at 3 time points during production. Production traits and egg quality traits were also determined. Data was analyzed using GLM: a significant difference was considered $P < 0.05$. There were no differences between the treatments in any measure at the start of the study ($P > 0.05$). The UV birds had lower CORT at 44 and 72 weeks of age (448.5 ± 70.5 ng/dl, $P = 0.03$; 1433.0 ± 163.4 ng/dl, $P = 0.05$), ASYM (4.39 ± 0.23, $P = 0.03$; 3.22 ± 0.18, $P = 0.004$) and HL (0.28 ± 0.02, $P = 0.002$; 0.31 ± 0.03, $P = 0.02$) at 44 and 72 weeks of age than the Control birds (699.4 ± 89.9 ng/dl; 1840.7 ± 124.5 ng/dl, 5.26 ± 0.33; 4.06 ± 0.22 and 0.41 ± 0.03; 0.42 ± 0.04). The UV birds righted faster during TI (299 ± 17 s, $P = 0.05$; 306 ± 19 s, $P = 0.04$) and flapped less intensely during INV (5.35 ± 0.17 flaps/sec, $P = 0.04$; 5.09 ± 0.17 flaps/sec, $P < 0.001$) compared with Control birds (347 ± 17 s; 361 ± 19 s and 5.90 ± 0.20 flaps/sec; 5.78 ± 0.13 flaps/sec) at 44 and 72 weeks of age. There was no difference between treatments in KLH, egg shell breaking strength, egg shell puncture strength, egg shell thickness, haugh unit, feed conversion, average egg weight at any of the time points ($P > 0.05$). While the total eggs laid per bird was not statistically significantly different the UV birds laid on average 4 more eggs during the study than the Control birds. These results demonstrate that rearing laying hens with UV light as part of the lighting spectrum can reduce their stress and fear response and once again indicate that it is important to use the correct lighting to optimize bird welfare and that having more natural lighting can help maximize that welfare and potentially improve production.

Key Words: layer, light, fear, stress, ultraviolet

350P The effect of feeding a seaweed extract during heat stress on broiler production and stress. E. Sobotik*, J. Nelson, and G. Archer, *Texas A&M University, College Station, TX.*

Heat stress is one on the main welfare issues that broiler chickens face and it can lead not only to decreased welfare but production as well. The seaweed *Ascophyllum nodosum* has shown the ability in other species to decrease body temperature and affect immune function. To determine if adding an extract of this seaweed into the diet of broiler chickens would decrease the effects of prolong heat stress a study was conducted. Broilers were fed the seaweed extract at a rate of 0.5 kg/metric ton of feed throughout a 42 d growout or just control feed. Half of each feed treatment was exposed to 2 weeks of heat stress (35°C for 16 h/day) starting at d28. Therefore, there were 4 treatments control non-stressed (CNS), control heat stressed (CHS), seaweed supplemented non-stressed (SWNS), and seaweed supplemented heat stressed (SWHS) To determine stress susceptibility bilateral asymmetry (ASYM, n = 60), heterophil to lymphocyte ratios (HL, n = 20), and plasma corticosterone concentrations (CORT, n = 20) were measured. Feed conversion, uniformity, and weight gain were also determined. Data was analyzed using GLM: a significant difference was considered $P < 0.05$. The CHS birds had higher CORT (211.9 ± 49.4 ng/dl), ASYM (1.99 ± 0.16) and HL (0.92 ± 0.19) than the CNS (113.1 ± 15.7 ng/dl, $P = 0.02$; 1.52 ± 0.11, $P = 0.004$; and 0.40 ± 0.06, $P = 0.001$), SWNS (123.5 ± 21.0 ng/dl, $P = 0.04$; 1.49 ± 0.10, $P = 0.002$; and 0.45 ± 0.07, $P = 0.003$) and SWHS birds (123.0 ± 20.6 ng/dl, $P = 0.04$; 1.45 ± 0.08, $P = 0.001$; and 0.61 ± 0.07, $P = 0.045$). There was no difference between treatments in final body weight, uniformity or feed conversion ($P > 0.05$). These results demonstrate that adding this seaweed extract to the feed of poultry can reduce their stress during a prolong heat stress event, though it had no effect on growth or feed conversion. This feed additive could be used to improve the welfare of poultry during heat stress events.

Key Words: seaweed, heat stress, broiler, production, welfare

351P Effect of a dietary phytogenic product on the production performance and coccidiosis in challenged broiler chickens. A. Plata* and A. Casarín-Valverde, *Nutec Group, El Marques, Mexico.*

A study was conducted to evaluate the effect of a phytogenic mixture contained in the product BIOADD, supplied by Euro-Nutec Premix, as an alternate solution against avian coccidiosis by reducing its effects on broilers production performance. A total of 2,400, day-of-hatch male broilers were fed with a standard diet supplemented with one of the 2 product concentrations for 49 d. The dietary treatments included (1) a negative control (NC), (2) a challenged positive control (PC), (3) Monensin at 110ppm (MoN), (4) BIOADD at 500 g/ton (Ba) and (5) BIOADD at 500 g/ton from d 0 to 28 and 375 g/ton from d 29 to 49 (Bb). Treatments 2 through 5 were challenged orally at d 14 with 1 mL of a cocktail containing a mixture of *E. acervulina, E. tenella* and *E. maxima*. Standard performance parameters were measured on d 0, 10, 21, 28, 42 and 49; as well as oocyst evolution and viability, and analyzed by one-way ANOVA with Tukey-Kramer Multiple-Comparison test. At

d 49, chicken's body weight (BW) of the Ba and Bb treatments were statistically higher ($P < 0.05$) from the NC treatment, but had no difference with statistical significance compared with the MoN treatment. There was no significant difference between the BIOADD treatments and MoN treatment for Feed Conversion Rate (FCR) and Daily Weight Gain (DWG), but BIOADD treatments were significantly different from the PC ($P < 0.05$). At d 21, oocyst count for *E. acervulina* in treatment Bb showed statistical significance ($P < 0.05$) compared with PC treatment with lower oocyst count, and no significant difference to MoN treatment. For *E. tenella* at d 21 BIOADD treatments had no statistical difference ($P > 0.05$) compared with PC and MoN treatments. In the case of *E. maxima* count at d 21 there was no statistical difference compared with PC and MoN treatments. All BIOADD treatments had numerical difference with PC. Johnson & Reid index for Ba showed a better numerical intestinal integrity at d 28 with 0.8, with no significant difference from other groups but with lower index than PC and MoN treatments with 2.6 and 1.8, respectively. Later, at d 35, Ba treatment had the lowest index with 0.16, being significantly different from the PC treatment ($P < 0.05$) with 0.51. Oocyst viability was significantly lower ($P < 0.009$) at d 35 for the groups Ba and Bb compared with MoN and PC group. In conclusion, the 2 concentrations of BIOADD were effective at the used dosage in alleviating the negative effects of a coccidiosis challenge, being the group Bb the most effective phytogenic group against *Eimeria*, presenting better numerical performance parameters, numerically reduced oocyst shedding and viability with no difference to the monensin treatment.

Key Words: phytogenic mixture, avian coccidiosis, oocyst count, intestinal integrity

352P The effect of stimulation electric shock on embryonic development in broiler chicks. S. Abdulateef[1], S. Rashid[2], and M. Mutlb*[1], *[1]University of Anbar, Anbar, Anbar, Iraq, [2]University of Sulaimani, Sulaimani, Kurdistan, Iraq.*

Chick embryo is like another animal's embryos, it needs more care because it grows outside the mother's body, so chick embryo require increasing feeding for development, this comes from utilization of nutrients and this resulting to consumption of substances inside the egg. During an embryonic development stage, the embryo undergoes seizures from hibernation, makes it unable on the utilization of food, so, for this reason, we inspired the idea, that embryo was shocked with an electric shock, makes it able broken the hibernation and thus aid in getting of feed. This study was conducted at the experimental field of the Department of Animal Production, College of Agriculture, University of Anbar, Iraq. 450 eggs were used (ross 308) distributed to 5 treatments each treatment 3 replicate. The treatments of the study were as following: T0 = control (without shock), T1 = Shocked (30) Millivolts (mV), T2 = Shocked (40) Millivolts (mV), T3 = Shocked (50) Millivolts (mV), T4 = Shocked (75) Millivolts (mV). A different Voltage apparatus was used for shocked the egg. After marking the eggs with a line of iron filings to ensure electrical conductivity, The eggs were shocked at the different times, 3 times a day started one day of hatching. The results showed: A significant increase ($P < 0.01$) in the length of the embryo, diameter of vascular region and the number of pairs of somites at 3 d of incubation for experimental treatments, a significant increase ($P < 0.01$) in the percentage of embryonic weight, and significant decrease ($P < 0.01$) in the percentage of albumin and the percentage of the shell at 7 d of incubation for experimental treatments. The significant increase ($P < 0.01$) in the percentage of embryonic weight and amniotic sac and liquid, and a significant decrease ($P < 0.01$) in the percentage of albumin and yolk, at 14 d of incubation for experimental treatments, significant increase ($P < 0.01$) percentage of embryonic weight and significant decrease ($P < 0.01$) in the percentage of yolk at the age of 17 d incubation for experimental treatments compared with control treatment.

Key Words: electric shock, embryonic, development, broiler, chick

353P A comparison of two methods of small-scale euthanasia in White Leghorn hens. A. Bigge*, C. Kreikemeier-Bower, S. Purdum, A. Reisbig, and K. Hanford, *University of Nebraska-Lincoln, Lincoln, NE.*

This pilot study, following institutional approval, compared 2 common methods for small-scale euthanasia (manual cervical dislocation and CO_2 displacement) for differences in apparent time of death and cessation of cardiac activity in eight 44-week old Bovans White Leghorn laying hens raised in 2 different housing systems (2 pairs of hens each from 2 aviaries and 2 cages). It was hypothesized that these parameters would differ between the 2 methods, with manual cervical dislocation resulting in the shortest time. It was also hypothesized that characteristics of cardiac activity after the procedure would differ. The hens were implanted with subcutaneous wireless telemetry devices 2 mo prior for another study already requiring euthanasia. Treatments were assigned randomly to individual hens within each pair, resulting in 2 hens per treatment combination in a split-plot design. Hens were captured from their cage or aviary and placed individually in cardboard boxes where they were transported to the procedure room. Wait time was consistent for all hens. Procedure time started upon signal from the handler after removing the hen from the box. Cervical dislocation was carried out manually. For the CO2 procedure the hen was placed in a clear plastic chamber (23"l × 16.25"w × 12.375"h) and gas was added at 30% displacement rate. Apparent time of death was determined to be the end of neuromuscular convulsions with no noticeable breathing. Euthanasia procedures were monitored by a veterinarian who evaluated the hens via auscultation of the heart immediately after the handler's confirmation, to provide a second means of confirmation. Each hen was monitored through a window in the procedure room while electrocardiogram (ECG) data continued to be collected for several minutes until cardiac activity ceased. Time points were recorded for the start of the procedure, handler confirmation, veterinarian confirmation, and end of ECG. ECG signatures indicating arrhythmia, tachycardia, and ventricular depolarization (VD) were identified. Data were analyzed using the Glimmix Procedure in SAS 9.4. There were no differences in total duration time (from procedure start to end of ECG) for method ($P = 0.43$) or housing type ($P = 0.94$), and no interaction between method and housing type ($P = 0.27$). There were also no differences in method for time to apparent death and time from apparent death to veterinarian confirmation ($P = 0.46$; $P = 0.63$) Heart activity continued well after the procedures, and ECGs differed qualitatively between the hens. Arrhythmia occurred in 5/8 hens, tachycardia in 3/8 hens, and VD in 6/8 hens.

Key Words: laying hen, euthanasia, electrocardiogram, cervical dislocation, CO_2 displacement

354P Influence of feed restriction on growth performance and gastrointestinal tract traits of two broiler strains differing in growth rate potential. G. Fondevila*, H. Mandalawi, L. Cámara, J. L. Archs, and G. Mateos, *UPM, Madrid, Madrid, Spain.*

The effects of feed restriction on growth performance and on the development of the proximal part of the gastrointestinal tract (GIT) were studied in broilers from 8 to 22 d of age. There were 4 treatments arranged as a 2 × 2 factorial with strain (Cobb 500 vs. Hubbard JV)

and feed restriction strategy [4 h darkness/d (R4) vs. 4 h darkness plus 4 extra hours with light but without access to the feeder (R8)]. Each treatment was replicated 6 times and the experimental unit was a cage with 8 chicks. All birds were fed a mash diet designed for slow growth broilers with 2,820 kcal AMEn/kg and 0.92% SID Lys. BW gain and DFI were determined from 8 to 18 d of age and FCR was calculated from these data. At 18 d of age, all birds (R4 and R8 groups) were kept under a 6 h dark period followed by 60 min of free access to the feed. Then, 2 birds per cage chosen at random were euthanized by asphyxiation in CO_2 atmosphere and weighed individually. The crop and the gizzard were excised and the weight (full and empty) and pH of the 2 organs were recorded. In addition, the fresh and the DM content of the digesta and the lactic acid concentration and the colonies of *Lactobacillus* spp. were measured in the crop. Main effects and the interactions of all growth performance and GIT traits were analyzed using the MIXED procedure of SAS. Cobb birds grew faster and had better FCR than Hubbard birds ($P \leq 0.001$). R4 broilers grew faster ($P \leq 0.001$) and tended to have better FCR ($P = 0.06$) than R8 broilers. The absolute (g) and relative (% BW) weight of the full ($P \leq 0.001$) and empty crop ($P \leq 0.05$), as well as fresh and DM content of the digesta, were greater for the R8 birds than for the R4 birds ($P \leq 0.001$). Furthermore, an increase in fasting time from 4 to 8 h had more pronounced effects on crop traits in the Hubbard than in the Cobb birds ($P \leq 0.01$ for the interaction). Lactic acid concentration was greater ($P \leq 0.05$) for the R8 birds than for the R4 birds whereas an opposite effect was observed for the Lactobacillus spp. colony count ($P \leq 0.01$). The length of the fasting period did not affect any of the gizzard traits studied but gizzard pH was higher for the Hubbard than for the Cobb birds ($P \leq 0.001$). Also, the empty and full gizzard were heavier, in absolute terms, for the Cobb than for the Hubbard birds ($P \leq 0.01$). In summary, increasing the length of the feed restriction period from 4 to 8 h increased the weight, digesta content, and lactic acid production of the crop, effects that were more evident in the Hubbard than in the Cobb birds. Compared with a fasting period of 4 h, fasting the birds for 8 h reduces BW gains in young broilers.

Key Words: broiler strain, crop, feed restriction, gizzard, organ pH

355P Effects of feed restriction on the development of the proximal part of the gastrointestinal tract in trained and non-trained broilers. Á. F. de Juan, G. Fondevila, J. L. Archs, L. Cámara, and G. Mateos*, *UPM, Madrid, Madrid, Spain.*

We studied the effects of feed restriction on the development of the proximal part of the gastrointestinal tract (GIT) in trained (TR) and non-trained (NTR) Ross 308 broilers. From 1 to 7 d of age, all birds were fed ad libitum a common cereal-SBM based diet in mash form. From 7 to 14 d of age, 5 different feeding strategies were used. There were a control group (AL) in which birds were fed ad libitum and 4 extra groups arranged as a 2x2 factorial with training (TR vs. NTR) and length of the fasting period (6 h vs. 8 h) as main effects. Fasting consisted in preventing the access of the broilers to the feeders from d 7 to 14, (TR birds had no access to feed during the fasting period whereas NTR birds had unrestricted access to feed from 7 to 13 d of age and then fasted on d 14 exclusively). Each treatment was replicated 6 times (a cage with 12 chicks). On d 14, at the end of the fasting period, all birds had free access to feed for 60 min. Then, 2 birds per cage were euthanized by CO_2 asphyxiation and weighed individually. The crop and the gizzard were excised and clampled to avoid digesta contamination and the weights (full and empty) and pH of both organs and the moisture content of the crop content were recorded. Data were analyzed as a completely randomized design. The following orthogonal comparisons were conducted 1) AL group vs. average of all feed restricted groups and 2) main effects and interactions among the 4 treatments arranged factorially. Treatment did not affect BW gain of the chicks from 7 to 14 d of age. In absolute (g) and relative (% BW) terms, the full crop was heavier in the restricted groups (TR and NTR) than in the AL group ($P \leq 0.01$). The fresh digesta content was lower for the AL fed group than for the average of the 4 restricted groups ($P \leq 0.01$). AL feeding reduced the pH of the crop ($P \leq 0.01$). In absolute ($P \leq 0.05$) and relative ($P \leq 0.01$) terms the weight of the empty crop was heavier for the TR birds than for the NTR or the AL fed birds. Gizzard contents were greatest for the AL birds ($P \leq 0.05$). The empty crop, after the 60 min of free access to feed, was heaviest for the TR birds ($P \leq 0.01$). An increase in the fasting period from 6 to 8 h increased the fresh digesta content of the crop by 25% but because of the high variability reported, the differences were not significant. In fact the length of the fasting period did not affect any of the GIT traits studied. In summary, fasting for 6 or 8 h changed the feeding behavior of young broilers, improving the development of the crop but with no effects on the gizzard.

Key Words: broiler, crop, feed restriction, gizzard

356P Light intensity preferences of broiler chickens under commercial conditions. D. Aldridge*, C. Scanes, C. Hayes, and M. Kidd, *University of Arkansas, Fayetteville, AR.*

There has been a growing interest in the relationship between light intensity and welfare for commercial broiler chickens. A choice approach was employed to determine whether there is a preference of Cobb 700 broiler chickens for light intensity. It was hypothesized that chickens would show a clear preference for light intensity. The test system consisted of 3 pens (0.91x1.23 m) each with waterers and a feeder. The pens were linked by a corridor with doorways to each pen. The corridor (0.31x3.66 m) did not have either waterers or feeders. There were 11 systems with the pens illuminated to 5 or 10 or 20 lx. The corridor was illuminated at 1 lx. From placement 30 straight run Cobb 700 chickens were reared in the system and lighting treatments began on d14. Numbers of chickens in the pens and corridor were discerned by remote video every 15 min during the first and last hour of the photophase as well as one random hour during the photophase on d14, d21, d30, d35 and d40. In addition, feed intake was measured between these ages. Overall, there were 22% more ($P < 0.05$) chickens in the 20 lx pens than 5 lx pens with the number intermediate in the 10 lx pens. Moreover, at d 14 and 21, there were 36 and 26% more ($P < 0.05$) chickens in 20 lx pens randomly through the day and 70 and 81% more ($P < 0.05$) birds in the 20 lx pens in the last hour of the photophase. At d 14, there were 3.1 ± (n = 11) 0.56 chickens per square meter in the corridor, despite the lack of feeders or waterers, compared with 7.0 ± (11) 0.77 SEM birds per square meter in the 5 lx pens and 8.8 ± (11) 0.86 birds per square meter in the 20 lx pens. The number birds per square meter increased with age ($P = 0.000295$) (slope = 0.106 number per day). At d 40, the number of birds per unit area were similar in the pens irrespective of light intensity and also in the corridor. Overall, there was less ($P < 0.006$) feed consumed in in 5 lx than 10 or 20 lx. It is concluded that Cobb 700 chickens exhibit some preference for 20 lx light intensity for those times of feeding (the first and last hour of the photophase). Moreover, with increasing age/size, there are increasing numbers of chickens in the corridor. This may reflect preference for lower stocking densities and/or birds migrating away from feeders/waterers toward low light intensities.

Key Words: lighting, preference, LED, broiler

357P Effects of feed restriction on the development of the proximal part of the gastrointestinal tract of two strains of broilers at 37 days of age. J. L. Archs[1], G. Fondevila[1], L. Cámara[1], B. González[2], and G. Mateos*[1], *[1]UPM, Madrid, Madrid, Spain, [2]Nutrave, S.A., Toledo, Spain.*

The development of the gastrointestinal tract (GIT) was studied in 2 broiler strains (Cobb 500 vs. Ross 308) after a feed restriction period of 6 h (dark period). The feeding program consisted in 3 commercial diets (0–10 d, 11–28 d, and 29–37 d of age) based on wheat and SBM. The light program consisted of 24 h of continuous light from 0 to 7 d and 18 h light from 8 to 37 d of age. There were 2 treatments (Cobb 500 vs. Ross 308) and 18 replicates (individual bird) per treatment. Each of the birds used were picked up from one of the 36 pens (30 broilers each) placed inside a commercial farm. After a 6 h dark period, birds had free access to feed and water for 60 min. Then, all birds were euthanized by CO_2 asphyxiation, and weighed. The crop and the gizzard were carefully excised, clamped to avoid digesta contamination, and weighed. Then, the 2 organs were emptied of any digesta content and weighed again and the absolute (g) and relative (RW, % BW) weight were recorded. In addition, the pH and the moisture content of the digesta were measured in both organs. The absolute and RW of the full and empty crop and of the fresh content of the digesta were higher ($P < 0.05$) for the Ross birds than for the Cobb birds. DM content and pH of the digesta, however, were not affected by broiler strain. The absolute and relative weight of the empty proventriculus, in absolute ($P = 0.081$) and relative ($P = 0.058$) terms, tended to be heavier for the Ross than for the Cobb birds. Fresh digesta content, however, tended to be higher for the Cobb tan for the Ross birds ($P = 0.088$). The weight and the characteristics of the gizzard digesta were little affected by bird strain. In summary, under the conditions of the current experiment, Ross 308 birds had heavier crops that had higher digesta content than Cobb 500 birds.

Key Words: crop, gastrointestinal tract, gizzard, strain

358P Diurnal photoperiod and in ovo lighting—Impact on hatchability traits of male Leghorn chicks. C. Ferreira dos Santos*[2], B. Rathgeber[1], T. Crowe[2], and K. Schwean-Lardner[2], *[1]Dalhousie University, Truro, NS, Canada, [2]University of Saskatchewan, Saskatoon, SK, Canada.*

The objective of this study was to determine the effects of photoperiod length during the incubation of Lohmann LSL-Lite eggs on spread of hatch, hatchability, embryo mortality and hatchling welfare. Four incubators (1502 Sportsman, G.Q.F Manufacturing Co., Savannah, GA) (windows blocked to eliminate outside light) were outfitted with LED lighting tubes (AGRISHIFT TLL, Once Inc., Plymouth, MN). A total of 300 eggs (100 eggs per hatch, 3 hatches per treatment; RCBD) were placed in each incubator, which were randomly assigned to one of 4 treatments: 0L(Light):24D(Dark), 6L:18D, 12L:12D or 18L:6D. Air temperature among the eggs was monitored using miniature data loggers (Hygrochron DS1923-F5 #, Maxim Integrated, San Jose, CA.). Infertile eggs and those with dead embryos were removed on d 9 and 18. The hatch was set to end 516 h after the start of incubation; starting 36 h before that end point, hatchlings were counted every 4 h. Post-hatch, 20 male chicks per incubator were sampled for navel quality scoring, body length, yolk sac free body weight, yolk sac weight, and organs were harvested (liver, heart and GI tract segments). Right and left femur, tibiotarsus and metatarsus were measured to determine fluctuating asymmetry (30 birds/incubator), and blood was collected for heterophil: lymphocyte ratio (H: L) analyses (15 birds/incubator). Data were statistically analyzed (SAS 9.4) with one-way ANOVA in a RCBD fashion. The spread of hatch was analyzed using a nonlinear regression model. Significance was noted when $P < 0.05$. Photoperiod/scotoperiod length during incubation affected the duration of incubation: 5% hatched chicks at 489.2^{a}, 485.6^{b}, 484.6^{b} and 481.6^{c} hour following start of incubation and 90% at 505.1^{a}, 503.5^{a}, 501.2^{ab} and 498.2^{b} hour following start of incubation for 0L, 6L, 12L and 18L, respectively. The photoperiod/scotoperiod length did not affect the spread of hatch. There was an effect on live body weight (41.82^{a}, 40.84^{ab}, 40.11^{ab} and 39.32^{b} grams; may have resulted from delayed weighing until all hatches were complete), and relative liver weight to body weight (2.13^{b}, 2.26^{ab}, 2.27^{ab} and 2.32^{a} percentage) for 0L, 6L, 12L and 18L, respectively. No impact of photoperiod/scotoperiod was noted for mortality, percentage of yolk free body weight and yolk sac weight to live body weight, navel closure, chick length, heart and GI tract segments relative weight to body weight, symmetry nor H:L ratio. To conclude, increasing the photoperiod length during incubation up to 18 h of light in a 24-h period reduces the incubation period with no adverse effects on hatchability, stress levels or quality of layer chicks.

Key Words: incubation, photostimulation, stress, spread of hatch

359P *N*-Acyl-phosphatidylethanolamine producing probiotic improves young broiler response to stress caused by individual housing and daily gavage. K. Moncada*, *Texas A&M University, Bryan, TX.*

Feed consumption, immune function and behaviors are key variables in poultry performance and welfare. Neuroactive *N*-acylethanolamines (NAE) are active in appetite control, behavior and immunity. We found tissue specific RNA expression of key NAE metabolizing enzymes was responsive to feed intake in breeder hens. The effects of these compounds in young birds are unknown. *N*-Acyl-phosphatidylethanolamines (NAPEs) are immediate NAE precursors and can be produced by probiotic *E. coli* Nissle 1917 carrying expression plasmid for the A. thaliana NAPE acyltransferase. We used this probiotic (pNAPE-EcN) and empty vector control bacteria (pEcN) for bulk probiotic production and live bird trials. Thirty Cobb 500 broiler chicks were housed in individual 1'x1' pens, with 12 never handled chicks housed in groups of 4 in 1'x1' pens. Chicks received a daily 0.5mL oral dose for 2 weeks to either pNAPE-EcN (10^4 cfu), pEcN (10^4 cfu) or 0.125% gelatin vehicle. Bodyweights were recorded daily, blood and livers were harvested at wk 2. Serum corticosterone was measured by ELISA. Liver NAPE content used LC/MS (https://doi.org/10.1172/JCI72517). Growth rates for individual birds were found to best fit $y = 0x^{p}$ where P = power function. Individual values for each group were averaged and difference in mean values compared using a *t*-test. The *t*-test revealed a statistical significance of 0.009 between the NAPE-EcN treated chicks and the EcN empty vector treated chicks in terms of growth rates, but no other statistical differences were shown between groups. The power values with their standard error for each treatment group are as follows; pNAPE-EcN = 7755 ± 196, pEcN = 7339 ± 248, Gelatin = 7553 ± 173. Corticosterone concentrations among singly housed chicks were similar and ~5-fold higher than never handled group housed chicks being 25.8 ± 3.4 ng/ml vs. 4.3 ± 0.7 ($P < 0.05$). Daily 1 min restraint while oral dosing combined with individual housing can be considered a high stress situation for young poultry. Probiotics in this setting did not provide a universal benefit, albeit provision of the pNAPE-EcN probiotic appeared to provide benefit compared with pEcN alone as birds were 34g (10%) heavier (NS) or gelatin as birds were 19.8g (5.6%) heavier (NS). Housing and handling methods must be carefully considered when evaluating probiotics aimed at improving welfare. AgriLife Research project #8738

Key Words: animal welfare, stress, broiler, feed restriction, broiler breeder

360P Incorporation of welfare assessment on layer performance test: 3 genotypes in 4 different housing systems. P. Regmi*[1], K. Anderson[2], D. Karcher[1], and D. Jones[3], [1]*Purdue University, Athens, GA,* [2]*North Carolina State University, Raleigh, NC,* [3]*USDA ARS, Athens, GA.*

Welfare quality of laying hens kept in conventional cages came under great scrutiny in the past decade and ultimately paved way for more extensive housing types such as the enriched colony cages and cage-free aviaries. Genotype can influence behavior and welfare; and information on the suitability of contemporary genetic strains of laying hens to different housing type is valuable to the producers. The aim of this study was to compare welfare parameters of 3 brown egg-type hens (O, P, and Q strains) in conventional (CC) and enriched cages (EN), cage-free (CF) litter and slat system, and a free-range (FR) system. Birds were either reared in place (CF and FR) or reared in cage systems and transferred at 16 wk. Welfare quality assessment was conducted on 10% hens per replicate at 15 wk age and then approximately every 8 weeks starting at 26 wk until the birds were 67 wk age. Welfare scores were collected on a binary scale and data were analyzed using non-parametric tests. Housing systems influenced keel fractures, keel deviations, foot condition and feather scores of hens. Keel fractures were more prevalent in FR hens (77.4%) compared with CC (35.8%), EN (40.9%) and CF (40.5%; $P < 0.05$). At 67 wk, CF also had lowest keel deviations than other systems ($P < 0.05$). Moderate food pad damage with hyperkeratosis was only present in the EN system (>80%). The cage systems (CC and EN) had broken feathers and featherless area <5 cm diameter whereas feather damage was very limited or non-existent in CF and FR ($P < 0.05$). Main effect of genetic strain was observed for keel fracture status and belly feather scores. Percentage of hens with keel fractures was greatest in strain 'Q' at 43 wk age whereas at 67 wk, fractures were more prevalent in 'Q' compared with 'P' while 'O' was intermediate ($P < 0.05$). At 67 wk, approximately 64% of 'Q' hens had moderate damage to belly feathers compared with 53% in 'O' and 35% in 'P' hens ($P < 0.05$). In conclusion, welfare parameters are influenced by environment, genetics and age of the hen. Other housing related management variables such as lighting, and underlying behavior differences among the genetic strains could be explored further.

Key Words: laying hen, genetic strain, housing, welfare quality

361P Effects of rearing and adult laying housing systems on medullary, pneumatic and radius bone attributes in 73-week-old Lohmann LSL lite hens. M. Neijat*, T. Casey-Trott, E. Kiarie, and T. Widowski, *University of Guelph, Guelph, ON, Canada.*

Consideration to minimize risks to skeletal integrity in laying hens throughout the egg production cycle underscores earlier interventions to stimulate bone development at the pullet stage. The objective of this study was to determine the interactive effect of a combined model of housing systems for rearing pullets and raising adult birds on bone quality as a measure of skeletal integrity. Lohmann Select Leghorn-Lite pullets were reared in either an aviary system (A) or conventional cages (C). At wk 16, the birds were transferred either to the same housing type through the adult stage (A-A or C-C, for rearing and adult phase, respectively) or to enriched system (A-E or C-E, respectively). In the rearing stage, C cages housed 16 pullets/cage (wk 0–6) and 8 pullets/cage (wk 6–16) with a space allowance of 145 and 290 cm^2/pullet, respectively; the rearing aviary housed 756 pullets/enclosure allowing 285 cm^2/pullet (wk 0–6) and 754 cm^2/pullet (wk 6–16). In the adult phase, C cages were 58.4 cm wide × 66.0 cm deep (482 cm^2/pullet, 8 pullets/cage). For adult A, a commercial multi-tier aviary provided a space allowance > 1000 cm^2/hen system with litter area. Enriched system housed 60 birds/cage at 750 cm^2/bird with perches, nest and scratch mat. Bone samples were collected from the adult phase (wk 73) and analyzed for bone quality indices including total bone weight (TBW), ash content of the leg (femur and tibia), wing (humerus and radius) and keel bones. Bird body weight was used as a covariate. Pullets and adult hens continuously reared in an A-A had the greatest ($P < 0.05$) TBW for medullary (femur and tibia) and pneumatic (humerus and keel) bones compared with other housing models. Femur-TBW was also improved ($P = 0.044$) for the C-E model compared with A-E or C-C but comparable to A-A. In addition, ash content was heavier ($P = 0.048$) for both A-A and C-E managed birds compared with the other rearing environments. Birds reared and raised in conventional housing (C-C) had the least score ($P < 0.05$) for TBW and ash content in all bone types. However, under the C-E model, the latter housing system (enriched) may act as a complementary or corrective measure to achieve improved bone quality. Regardless of the housing system, bone breaking strength (BBS, kg) was significantly correlated ($r = 0.60$; $P < 0.01$) to the amount of ash (% of bone weight) and TBW, particularly for humerus and tibia. The results provide an insight on the impact of type of housing system in the rearing stage, early in life, on bone ash/mineral composition, thereby reflecting a healthy bone development and skeletal integrity to support laying hens later in the production cycle.

Key Words: pullets, laying hen, housing system, bone composition, bone strength

362P Assessment of computer science and cybersecurity skills of undergraduate students interested in food safety. K. Feye*, L. Meyer, H. Lekkala, D. Thompson, and S. Ricke, *University of Arkansas, Fayetteville, AR.*

Automation is coming and will enable not only the ability to increase poultry processing line speeds, but collection of "big data." This data can be collected en masse, stored, analyzed, and used to improve food safety, quality, enhance traceability and also be used for risk assessment. Therefore, development of these tools are essential for the poultry industry. However, as this technology is implemented, hackers will emerge and present danger to the poultry industry. As a result, there is a need for computer science (CS) trained food safety professionals in poultry production that specialize in data analysis and can develop cybersecurity strategies. Before specific educational programs can be developed, assessing the CS knowledge of emerging professionals is essential. The objective of this study was to assess the students for CS and cybersecurity skills and interests of students by using an anonymous surveying of 62 non-major undergraduates enrolled in an Introduction to Food Science course. Students ranked their CS and cybersecurity skills from 1 through 5, with 1 being the least familiar and 5 representing expert level knowledge. Collected responses were analyzed using One-way ANOVA with pair-wise contrasts with the Tukey correction for multiple comparisons ($P < 0.05$). The average student was familiar with common avenues of food safety exposure such as television (3.28), Internet (3.49), cooking shows (3.21), farms, but had a statistically significant unfamiliarity with radio (2.2), newspapers (2.2), home and garden (2.25), and with farmers' markets (2.61). While many students had previously gained home economics and restaurant experience, there was less experience with on-site farm work (9), processing plant (0), or state fair (0) ($P < 0.05$). Participants demonstrated a significant understanding of basic CS skills, such as word processing (3.9), emails (4.3), and (3.9). However, more advanced CS knowledge, such as Linux (1.3), hacking (1.9) was less familiar ($P < 0.001$). This survey finding likely corresponds with a lack of advanced CS coursework. Most students had taken some form of a computer CS class (n = 19), while only one student had taken more advanced coursework ($P < 0.05$). Students were less familiar with Botnet (1.97), denial of service attack (2.1) and identity theft ($P < 0.05$), ransomeware (2.3), as compared with identity theft (3.1) ($P < 0.05$). Undergraduate web series interest in food science have higher CS skills in common applications and threats, but lower CS skills in areas related to software development and security. Therefore, to meet the future technological demands, specific course-work is required to improve prospective student CS and cybersecurity competency.

Key Words: cybersecurity, food safety, computer skills, undergraduate student, food safety

363P The effect of genetic selection on gene expression in the developing somatotropic axis of broilers. L. Vaccaro*[1], P. Sinpru[2], K. Brady[3], Y. Chaiseha[2], T. Porter[3], and L. Ellestad[1], *[1]University of Georgia, Athens, GA, [2]Suranaree University of Technology, Nakhon Ratchasima, Thailand, [3]University of Maryland, College Park, MD.*

Over the past 50 years, genetic selection has led to improvements in growth and feed efficiency in modern broiler chickens. However, the biological basis behind this improved performance is not fully understood. The objective of this study was to evaluate effects of commercial selection on gene expression in neuroendocrine and somatic components of the developing somatotropic axis during embryogenesis. To do this, gene expression in a modern commercial line (Ross 708) was compared with that in Athens Canadian Random Bred (ACRB) birds, a legacy population that has remained unselected since 1956. Hypothalami, anterior pituitary glands, and liver were collected from 12 embryos of each line on embryonic days (e) 10, 12, 14, 16, and 18, and embryo weights were recorded. Visual sex identification was confirmed using molecular sexing by PCR, and total RNA was extracted from each tissue from 4 male and 4 female embryos (n = 4). Expression levels of hormones and their receptors regulating growth and body composition were measured using real-time quantitative PCR and normalized to GAPDH (hypothalamus, liver) or PGK1 (pituitary). Data were analyzed for significant effects of line or the line-by-age interaction within each sex using a 2-way ANOVA followed by the test of least significant difference. In males, Ross embryos were heavier than ACRB on e14-e18 ($P < 0.01$), while in females this difference was not apparent until e18 ($P < 0.01$). In the hypothalamus, growth hormone releasing hormone (GHRH) mRNA was higher in Ross females on e12 and higher in ACRB females on e18 ($P < 0.05$), and somatostatin mRNA was higher in ACRB in both sexes ($P < 0.05$). There was no significant difference in expression of pituitary growth hormone (GH) mRNA, but levels of GHRH receptor were higher in Ross embryos regardless of sex ($P < 0.01$). In females, GH secretagogue receptor was higher in ACRB at earlier ages (e12-e14) and Ross at later ages (e16-e18), and pituitary adenylate cyclase-activating polypeptide receptor was higher in Ross embryos at later ages (e16-e18; $P < 0.05$). In female embryos, liver insulin-like growth factor 1 mRNA was higher in Ross ($P < 0.05$), and they also tended to have higher levels of thyroid hormone receptor β ($P = 0.0665$). In male embryos, GH receptor mRNA appeared higher in ACRB than Ross ($P = 0.0718$). These results suggest that gene expression for components of the GH axis during embryonic development have been altered due to commercial selection, and these differences may contribute to the improved performance seen in modern broilers.

Key Words: selection, growth, metabolism, development

364P Effects of *FMO3* gene expression and FMO3 enzyme activity on the fishy odor of duck eggs. X. Li*, Y. Guo, J. Song, X. Shi, G. Xu, and J. Zheng, *China Agricultral University, Beijing, China.*

Fresh duck eggs have a strong fishy odor. Previous studies had revealed that fishy odor in human or other animals were caused by trimethylamine (TMA). We inferred that the fishy odor of duck eggs was also due to TMA. TMA is normally oxidized by flavin monooxygenase 3 (FMO3) enzyme into trimethylamine-N-oxide (TMAO). However, we found no *FMO3* gene mutations related to TMA production. This study is aimed at determining the effects of *FMO3* gene expression and FMO3 enzyme activity on the metabolism of TMA. Sensory evaluation of duck and chicken egg yolks (fishy levels: 0–5) were evaluated, TMA content was determined using Headspace gas chromatography. The absolute *FMO3* gene expression and FMO3 enzyme activity of livers (measured in vitro represented by TMAO production) in ducks, dwarf laying hens (including T329S mutations: AA, AT, and TT), and white leghorn hens were analyzed. The *FMO3* gene expression of ducks in the high and low groups (ducks which yielded high and low TMA content in eggs, respectively) were also compared. Statistically significant differences were performed using one-way ANOVA (outliers were removed). The sensory evaluation results showed that the duck eggs scored 1 to 5 points, while the scores of chicken eggs ranged from 0 to 2 points. TMA content in duck egg yolks (0.57 ± 0.19 μg/g) was generally higher than that of chickens (0.47 ± 0.21 μg/g). The absolute *FMO3* expression levels in ducks, dwarf laying hens (AA, AT, TT), and white leghorn hens were 0.124 ± 0.0615, 0.0305 ± 0.0113 (AA), 0.0279 ± 0.019 (AT), 0.0513 ± 0.0423 (TT), and 0.0232 ± 0.00538, respectively. The absolute expression level of *FMO3* gene in Jingjiang ducks was significantly higher than that in the other 4 groups ($P < 0.05$). However, the relative *FMO3* expression level of ducks in the high (13.1 ± 6.67) and low (7.43 ± 8.59) groups showed no significant difference ($P > 0.05$). The FMO3 enzyme activities in ducks, dwarf laying hens (AA, AT, TT), and white leghorn hens were 1.94 ± 1.13 μg/g, 4.64 ± 1.38 μg/g (AA), 2.78 ± 1.18 μg/g (AT), 1.26 ± 1.26 μg/g (TT), and 6.35 ± 2.15 μg/g, respectively. The FMO3 enzyme activity of white leghorn was significantly higher than that of ducks and dwarf laying hens (AT and TT) ($P < 0.05$). In summary, we found that both the fishy level and TMA content in duck egg yolks were higher than that in chickens. It supports the hypothesis that TMA contribute to the fishy taint of duck eggs. Though the *FMO3* expression in duck liver was significantly higher, the high TMA content in duck eggs and the fact that the *FMO3* expression level in the high and low groups showed no significant difference indicated that *FMO3* gene contributed little to the metabolism of TMA on the transcription level.

Key Words: fish odor, TMA, FMO3, gene expression, enzyme activity

365P Whole genome resequencing to determine copy number variations associated with stress responses in Japanese quail. B. Khatri*, S. Kang, S. Shouse, N. Anthony, W. Kuenzel, and B. Kong, *University of Arkansas, Fayetteville, AR.*

Copy number variation (CNV) is a major driving factor for genetic variation and phenotypic diversity in animals. To detect CNVs and understand genetic components underlying stress related traits, we performed whole genome re-sequencing analysis in High Stress (HS) and Low Stress (LS) Japanese quail lines. Blood samples (3mL) were collected from 20 adult male birds each from HS and LS lines. Genomic DNA was purified from each sample and 12 samples showing highest quality per line were pooled to represent each line. For whole genome sequencing, library preparation and Illumina sequencing for the pooled DNA samples were performed using Illumina HiSeq 2 × 150 bp paired end read technology. Raw sequencing data were aligned using reference based assembly on the quail genome sequences and performed bioinformatics analyses. Our bioinformatics analyses were then complemented with experimental validation using real time quantitative PCR (qPCR). As results, the depth of coverage for re-sequenced data reached to 41.4x and 42.5x respectively. Using read-depth approach and CNVnator tool to detect CNVs in the aligned data sets, we found 262 (235 deletions

and 27 duplications) and 168 (148 deletions and 20 duplications) CNV regions (CNVRs) affecting 15.20 Mb (1.6%) and 18.17 Mb (1.9%) of reference genome in HS and LS birds respectively. Using BEDOPS tool and custom bash script, we extracted genes from CNVRs and found 454 unique genes in HS and 493 in LS birds associated with CNV. Results of Ingenuity Pathway Analysis (IPA) showed that the CNV genes were significantly enriched to phospholipase C signaling, neuregulin signaling, reelin signaling in neurons, CD27 signaling in lymphocytes, endocrine and nervous development, humoral immune response, and carbohydrate and amino acid metabolisms in HS birds. In LS birds, signaling pathways including cell-mediated immune response and protein and lipid metabolisms were enriched with CNVgenes. We identified sets of genes affected by CNVs in HS and LS birds, most notably involved in nervous/ endocrine systems development and humoral/ cell-mediated immune response. The findings in this study suggest CNV genes identified in HS and LS birds can be candidate marker genes responsible for stress responses in birds. This work was supported by USDA multistate project, NC1170, Advanced Technologies for the Genetic Improvement of Poultry and Arkansas Agricultural Experimental Station.

Key Words: Whole genome re-sequencing, Copy number variation, Restraint Stress, Japanese Quail

366P Identification of an autosomal dominant achondroplasia in White Pekin ducks. G. Barbato*[1] and Z. Lowman[2], *[1]Stockton University, Galloway, NJ, [2]Joe Jurgielewicz and Sons LLC, Shartlesville, PA.*

While sorting several hatches of a pedigreed population of commercial White Pekin ducks, one of us (GFB) noticed a large number of birds with what appeared to be short legs. Subsequently, we measured the shank length of > 10,000 42d old ducks from a pedigreed population selected for growth. There was a clear bimodal distribution of shank lengths with the mean of each distribution being 84 and 90 mm. Body weight, however, did not show a concordant distribution, with all birds averaging 7.1 ± 0.85 lbs. Further, there were no sex differences in shank length. Suspecting that the bimodal distribution suggested a mono- or oligogenic origin, we proceeded to establish purebreeding families (i.e., purelines) and perform a traditional genetic analysis consisting of F_1, F_2 and backcrosses. All subsequent shank length and body weights were measured at 42 d of age, although independent observations have concluded that the dwarfing effect is present from hatch to sexual maturity. After 3 generations, 8 pureline families were created (4 short- and 4 long-shanked). These families produced 3rd generation progeny having short (77 ± 2 mm) and long (93 ± 2 mm) shanks with nonoverlapping distributions. In the subsequent 2 generations, F_1, F_2 and backcrosses were made to determine the mode of inheritance of shank length. The initial F_1 reciprocal crosses had shank lengths of 83 ± 3mm, which was not significantly different from the short-shanked families of that generation ($P = 0.43$). Chi-squared analysis of the F_2 progeny in the next year supported the observation of the dominance of short shanks over long shanks - resulting in the expected 3:1 Mendelian ratio ($\chi^2 = 1.20$; df = 2). Backcross populations were also consistent with the hypothesis of a dominant gene resulting in short shanked ducks. Of particular note was the B2 backcross (consisting of F_1 progeny crossed to purebred long shanked ducks), which resulted in a 1:1 ratio of short- and long-shanked ducks. As there are other dominant achondroplasias that have been identified in vertebrates, we suggest the designation "*Ach"* as the gene name for dominant achondroplasia in ducks.

Key Words: Pekin duck, growth, dwarf, achondroplasia

367P Selection for body weight at 14d in White Pekin ducks. G. Barbato*[1] and Z. Lowman[2], *[1]Stockton University, Galloway, NJ, [2]Joe Jurgielewicz and Sons LLC, Shartlesville, PA.*

Marks (1980) and, later, Ricklefs (1985) suggested that the major improvement due to selection for growth in poultry occurred during the first 14d posthatch. This hypothesis was tested and found to be true via a double-divergent selection experiment for 14 or 42d growth by Barbato (1991). As the growth curve of ducks is very similar to that of broiler chickens, we hypothesized that selection at 14d would provide similar benefits to duck populations as it did in chickens. This report includes the results of 18 generations of selection for 14d body weight in a commercial population of White Pekin ducks. Initially, 3 hatches of 11,000 – 12,600 ducks from a commercial pureline were weighed at 14 and 42 d of age and parents selected with a selection intensity of 1.40. The population was maintained by mating 48 drakes with 5–6 females per generation. Approximately 8,000 progeny were produced over 6–8 hatches within each generation. During the first 6 generations of selection an unselected control line was maintained. The direct genetic response was 19 g/generation ($P < 0.001$; $R^2 = 0.85$). Over that time, the 42d weights increased 37.5 g/generation ($P < 0.001$; $R^2 = 0.54$). The realized heritability of 14d weight was 0.45 ± 0.09, which was in the same range as that reported by Barbato for chickens. The phenotypic correlation ($r_{PP'}$) between 14d and 42d body weight was 0.80 while the genetic correlation ($r_{GG'}$) was 0.57. Again, reinforcing the hypothesized relationship between 14d and 42d growth. Overall phenotypic progress across 18 generations was 61 g/generation ($P < 0.001$; $R^2 = 0.58$). The selected line continues to respond (phenotypically) to selection, although we are unable to estimate direct genetic response due to the loss of the control line. The line is now a critical component of a commercial crossing scheme.

Key Words: duck, selection, heritability, early growth, body weight

368P Femur evaluations of broilers from random bred lines of the 1990s and 2010s. E. Briggs[1], E. Kneeland[1], S. Orlowski[2], N. Anthony[2], and K. Tarrant*[1], *[1]Fresno State, Fresno, CA, [2]University of Arkansas, Fayetteville, AR.*

Intensive selection pressure in fast-growth broilers has resulted in continuous gains in economically important traits. Two random bred (RAN) populations representing the genetic potential of broilers from 1995 and 2015 were evaluated to determine how such selection has influenced femur parameters of broilers. Broilers were hatched from the 1995 RAN and 2015 RAN and raised straight run for 6 weeks with access to ad libitum feed and water. Eighty individuals from each line were sampled over 4 time points (d 0, d 14, d 28, and d 42). Live weight and left leg parameters were recorded, including: leg, thigh, and femur weights. Femur length, femur neck, proximal femur widths (frontal and sagittal), and distal femur widths (frontal and sagittal) were measured. A *t*-test was conducted through JMP to compare means between lines at different ages. As expected, due to genetic selection gains in body weight over time, both male and female live weights were significantly larger in the 2015 RAN line ($P < 0.01$). The d 42 2015 RAN mean male weight was 462 g larger than 1995 RAN males, while 2015 RAN mean female weight was 399 g larger than 1995 females. Left leg weight ($P = 0.05$), thigh weight ($P = 0.01$), and femur weight ($P = 0.001$) were significantly larger at d 0 in the RAN 1995 line, when compared with the 2015 RAN line. By the time d 14 was reached, these parameters were significantly larger in 2015 RAN line broilers compared with the 1995 RAN ($P = 0.04$, $P = 0.03$, $P = 0.02$, respectively). This trend continues

in the d 28 and d 42 time points. Dimensional measures indicated no difference was present at d 0 between the means in the femur neck (P = 0.48), proximal femur width - frontal view (P = 0.89), proximal femur width – sagittal view (P = 0.55), and distal femur width - frontal (P = 0.92), and distal femur width – sagittal (P = 0.81); however, the femur length was smaller in the RAN 2015 line (P < 0.001). To maintain a body frame that can withstand the physiological pressures associated with intensive selection in fast-growth broilers, selection practices incorporate genetic selection of skeletal integrity. Maintenance of leg health throughout growout remains critical, as lameness negatively impacts broiler welfare and economics. Interestingly, the 1995 RAN line broilers supported more robust femur and thigh parameters at d 0. Nutritional allocation during embryonic development may be the cause of the smaller leg in the 2015 RAN line. Identifying d 0 as a time point for intensive selection of leg health traits may be beneficial in improving leg health in modern broilers.

Key Words: broiler, genetic, selection, leg health, femur

369P Influence of dietary spray dried plasma on fecal microbiota in broiler chickens. Y. Jababu*[1], C. Blue[1], P. Ferket[2], S. Ibrahim[1], R. Gyawali[1], and Y. Fasina[1], *[1]North Carolina A&T State University, Greensboro, NC, [2]North Carolina State University, Raleigh, NC.*

Intestinal microflora constitutes a dynamic ecosystem that is essential to the health of the chicken. Consequently, it is imperative to determine the effect of feed additives on intestinal microbiota composition. Spray dried plasma (SDP) is an emerging immunomodulatory feed additive due to its constituent growth factors and functional proteins. A 28-d experiment was conducted to determine the effect of dietary SDP on selected bacterial populations. Day-old (240) Ross 708 male chicks were obtained from a commercial hatchery, weighed, and randomly assigned to 6 dietary treatments. Treatment 1 (CX) consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without SDP. Treatment 2 (MX) consisted of chicks given unmedicated corn-SBM basal into which Bacitracin methylene disalicylate (BMD) was added at 0.055g/kg diet. Treatments 3 (SP1), 4 (SP2), 5 (SP3), and 6 (SP4) consisted of chicks given unmedicated corn-SBM basal into SDP was added at 10, 20, 30, and 40 g/kg diet, respectively. Each treatment consisted of 4 replicate pens, with each pen housing 10 chicks. On d 19, fresh fecal samples were collected from each replicate pen in each treatment, and subjected to appropriate microbiological procedures. Specifically, fecal dilutions were plated onto Brain Heart Infusion (BHI), McConkey, deMan–Rogosa–Sharpe (MRS), and modified Bifidobacterium Iodoacetate Medium-25 (mBIM-25) agar for the enumeration of total bacteria, *Escherichia coli*, *Lactobacillus* spp., and *Bifidobacterium* spp., respectively. The agar plates were incubated for 24 to 72 h, and colonies were counted thereafter. Results showed that chicks in SDP-supplemented treatments (SP1, SP2, SP3, and SP4) had a lower population ($P < 0.05$) of *E.coli* (a pathogenic bacteria), compared with the medicated MX treatment. There were no differences among treatments ($P > 0.05$) in the population of *Lactobacillus* spp. (healthy bacteria strain) among treatments. The population of *Bifidobacterium* spp. (healthy bacteria strain) for SP2, SP3, and SP4, were comparable to that of MX treatment. It was concluded that dietary inclusion of SDP at levels between 30 and 40 g/kg diet reduced *E. coli* population, and showed bifidogenic potential.

Key Words: broiler chick, spray-dried plasma, fecal microbiota, *Escherichia coli*, *Bifidobacterium* spp.

370P Evaluation of antibiotic alternatives in *Eimeria* vaccinated broiler chickens. M. Pineda*[1], D. Zhao[1], S. Gurung[1], D. White[1], A. McElroy[1], Y. Farnell[1], M. Kogut[2], K. Genovese[2], J. Lee[1], B. Mozisek[3], I. Alvarado[3], N. Evans[4], and M. Farnell[1], *[1]Texas A&M University, College Station, TX, [2]USDA-ARS, College Station, TX, [3]Merck Animal Health, Elkhorn, NE, [4]PMI Nutritional Additives, Arden Hills, MN.*

Coccidiosis is a prevalent disease in the broiler industry. The pathogen costs the US poultry industry an estimated $90 million annually due to reduced weight gain and increased feed conversion. Seven major species of *Eimeria* infect different locations within the poultry gastrointestinal tract causing diarrhea and weight loss due to malabsorption. Prevention and treatment of coccidiosis has previously relied on in-feed anticoccidials, however resistant strains of *Eimeria* have developed. Alternatives to coccidiostats include vaccination, prebiotics, probiotics, phytogenics and nutritional strategies. We hypothesized that these antibiotic alternatives would ameliorate any negative effects that may be associated with live coccidiosis vaccination and improve broiler performance. Day-of-hatch male broiler chicks were raised on used litter to evaluate the effect of a live commercial coccidia vaccine when combined with antibiotic alternatives during a 42 d grow out period to evaluate bird performance and oocyst cycling. Broiler chicks were assigned to 9 treatments with 11 replicates per treatment (28 birds per replicate) consisting of the following: 1) negative control, 2) Coccivac B52 vaccine, 3) prebiotic + probiotic + phytogenic, 4) short and medium chain fatty acid + phytogenic (0–14d) and then phytogenic alone (15–42d), 5) medium chain fatty acid (MCFA) alone, 6) treatment 3 + vaccine, 7) treatment 4 + vaccine, 8) treatment 5 + vaccine, 9) bioshuttle program with vaccine and salinomycin. Pen weights, feed intake and lesions scores were evaluated on d 14, 28, and 42. Feed conversion ratios were calculated as grams of feed/grams of weight gain and corrected for mortality. Oocysts per gram of fecal collection were determined on d 7–8 and 19–20. Macroscopic intestinal lesion scores were evaluated using the Johnson and Reid method. Microscopic lesion scores were quantitated for *E. maxima*. Data were analyzed via a one way ANOVA with significant differences determined at $P < 0.05$ and separated using Duncan's multiple range test. There were no significant differences in weight gain, feed conversion ratio, oocysts per gram of fecal material, or intestinal lesions at the termination of the study. The effects of the antibiotic alternatives combined with the coccidiosis vaccine did not have a negative effect on broiler performance or gut health. The trial was conducted under optimal growing conditions with relatively low lesion scores. Future studies are needed to reevaluate these products in broilers exposed to increased stressors due to housing environment and microbial challenge.

Key Words: coccidiosis, broiler, antibiotic alternative, vaccination, gut health

371P Induction of interleukin-10 expression in chicken intestinal epithelial cells stimulated with *Clostridium perfringens.* Y. Lee*[1], H. Lillehoj[1], W. Kim[1], and S.-J. Lee[2], *[1]USDA-ARS, Beltsville, Maryland, [2]Kangwon National University, Chuncheon-si, Gangwon-do, Korea.*

Interleukin (IL)-10 is an anti-inflammatory cytokine which regulates host innate immune response. Besides T cells which are the major source of IL-10, the intestinal epithelial cells (IECs) also have been shown to express IL-10 to maintain the epithelial integrity in mammals. In chickens, there is no reported study on the expression of IL-10 in chicken IECs. In this study, we treated chicken IECs with *Clostridium perfringens* (CP), a causative agent of necrotic enteritis (NE). NE is caused by virulent strains of CP which induce severe local inflammatory response in chicken intestinal mucosa. In the present study, chicken IECs were stimulated with either heat-killed CP (HK-CP) or supernatant from CP culture which were collected at different time points and dosage levels. The expressions of chicken IL-10 and chicken IL-6 (an inflammatory marker) were monitored by quantitative real-time polymerase chain reaction (qRT-PCR). In addition, we developed an antigen capture assay using the newly developed mouse monoclonal antibodies against chicken IL-10, to quantify IL-10 protein production in CP-stimulated IECs. Compared with non-stimulated control, the mRNA expressions of IL-10 were highly induced in IECs stimulated with HK-CP and the supernatant from CP culture. Interestingly, the supernatant from CP culture contained CP toxins and induced higher level of IL-10 compared

with HK-CP indicating that CP toxins may play a role in IL-10 production. Additionally, the protein concentration of IL-10 measured by the antigen capture assay agrees with the mRNA expression. The results of present study provide a new insight on early host immune response to CP in NE infection.

Key Words: interleukin-10, chicken, intestinal epithelial cell

372P Reducing gut lesions caused by bacterial enteritis with a specific blend of esterified fatty acids. K. Kozlowski[2], A. Lauwaerts*[1], and J. Jankowski[2], *[1]Proviron Industries NV, Hemiksem, Belgium, [2]University of Warmia and Mazury in Olsztyn, Olsztyn, Poland.*

Modern turkey breeds remain vulnerable to enteric diseases and bacterial challenges such as *E.coli*, *Salmonella* and *Clostridium*. Therefore it is essential to create a healthy intestine from the very start and to maintain optimal gut functions under high pressure conditions throughout the whole growth period. The efficacy of Optigut, a specific combination of esterified butyric and lauric acid produced by Proviron Industries, on gut health and overall performance was tested in turkeys. A total of 1400 healthy one-day-old turkey females Hybrid Converter were randomly divided into 4 treatment groups with 7 replications each. The experimental groups comprised of a negative control group (NC), fed a basal, commercial diet; a positive control group (PC), fed the same basal diet, but receiving antibiotics via the drinking water throughout the entire experiment and 2 treatment groups receiving a feed either a high (HD) or low (LD) degressive supplementation of Optigut. All birds were individually orally inoculated with a bacterial and *Eimeria* cocktail during 3 consecutive days from the age of 19 d. They were reared up to 105 d. On d 26, 3 animals per pen were evaluated for intestinal gut health and coccidiosis. A macroscopic intestinal lesion scoring system, as an assessment of the macroscopic pathology and physical damage caused by bacterial enteritis, was combined with lesion scoring for coccidiosis, as subclinical coccidiosis plays a major role in the development of bacterial enteritis. Data of various parameters were analyzed with a one-way ANOVA procedure with significance level at $P = 0.05$.. The LD treatment shows a near-significant trend for a lower coccidiosis scoring compared with the HD, as well as for a lower bacterial enteritis scoring compared with both HD and NC. Moreover, flaccidity in LD and PC is significantly lower than in HD groups and the LD groups show a trend toward lower inflammation compared with HD and PC. Higher butyrate concentration reduces the mucin production. A thinner mucosa layer may allow parasites and pathogenic bacteria, like *Clostridium perfringens*, to reach the gut wall easier and inflict more damage. Glycerol monolaurate reduces immune cell proliferation at high concentrations, whereas it increases the proliferation of T-cells at lower concentrations. The impact on flaccidity may be due to overstimulation of the immune system at higher concentration of glycerol monolaurate. Also antibiotics perturb commensal microbial communities and affect susceptibility to infection by intestinal pathogens and development of inflammatory bowel diseases.

Key Words: turkey, coccidiosis, bacterial enteritis, butyrate, monolaurate

373P Baseline metabolic capacity of peripheral blood mononuclear cells from multiple chicken genetic lines. M. Meyer*, J. Jespersen, and E. Bobeck, *Iowa State University, Ames, IA.*

Baseline cellular metabolism preference is a reflection of environment, nutrition, genetics, and individual variation in poultry. We hypothesized that the use of an advanced metabolic assay (Seahorse XFe Analyzer, Agilent) to compare baseline metabolic rates would yield different results among different genetic strains of birds due to trait selection for growth (broiler) or production (layer). Since most Seahorse Assay published work uses immortal cell lines or non-poultry species, we first generated foundational immune cell metabolic data in laying hens and broilers by titrating to determine preferred substrate, pathway, and optimal cell seeding concentration using fresh peripheral blood mononuclear cells (PBMCs). The optimal concentration for all strains was determined to be 3 million cells/well based on the necessity for a cell monolayer. The Cell Energy Phenotype Test (Agilent) measured the baseline mitochondrial respiration of the cell (Oxygen Consumption Rate; OCR) and the baseline glycolytic activity (Extracellular Acidification Rate; ECAR) to determine metabolic capacity and preferred pathways. Metabolic preferences of 9 lines of birds, including modern commercial laying hen (Bovans White) and broiler (Ross 308) lines, along with Iowa State University's inbred Fayoumi (LM 15.2), broiler (BR), Spanish (SP), and Leghorn (GHS6 and L8) lines, and advanced intercrosses of broiler and Fayoumi (BRX 15.2) and broiler and Leghorn (BRX 6) lines were analyzed. OCR and ECAR means were compared using the mixed procedure of SAS followed by a Tukey-Kramer adjustment. Results indicate that the effect of line on ECAR was significant ($P = 0.0241$) with the difference in means of Ross 308 and Bovans White PBMCs approaching significance ($P = 0.0601$). The effect of genetic line on baseline OCR was not significant ($P = 0.0878$), a result we have seen previously when comparing commercial broilers and laying hens only. ECAR is a measurement of glycolysis while OCR measures mitochondrial respiration, therefore, our data indicate that selection of lines for vastly different traits over time has had a profound effect on the likelihood of using a glycolytic pathway vs. mitochondrial respiration. Since modern Ross 308 broilers and Bovans White laying hens showed the greatest difference in baseline ECAR, these 2 lines were compared in a metabolic pathway inhibitor drug challenge (FCCP and Oligomycin), which resulted in no differences in metabolic capacity. These results indicate that while these lines of birds differ in their baseline metabolic pathway preferences, their capacity to use non-preferred substrates to respond to an inhibitor challenge is not affected.

Key Words: PBMC, metabolism, broiler, laying hen, genetics

374P Assessment of a NSPase enzyme, Natugrain TS, dose titration on jejunum viscosity, IDE and necrotic enteritis lesion scores on birds challenged with coccidiosis vaccine and clostridium perfringens and 28-day bird performance on corn/soy diets. A. Troescher*[2] and M. Coelho[1], *[1]BASF Corporation, Humble, TX, [2]BASF SE, Lampertheim, Germany.*

The objective of this study was to evaluate the efficacy of a NSPase enzyme, Natugrain TS, dose titration on jejunum viscosity (with a Brookfield digital viscometer), apparent ileal digestibility of energy (IDE) (with a TiO_2 marker) and NE lesion scores (0–5 visual) on birds challenged with coccidia vaccine and Clostridium perfringens (Cp) and 28-d bird performance on corn/soy diets. A total of 480 male Cobb 500 broilers were used in a randomized complete block design (8 birds/cage × 6 Natugrain TS doses × 10 replicates). Feed was pelleted at 89C, 45 s., birds were coccidiosis vaccinated (Coccivac B52) at day1 and α toxin and netB-positive Cp administered at d19, 20 and 21. The Natugrain TS doses were 0 (NC), 50, 75, 100, 125 and 150 g/MT. IDE, jejunum viscosity, jejunum lesion scores and performance were measured at d28. Data were subjected to one-way ANOVA. Additionally, linear and quadratic regression analysis were performed. Birds fed 125g Natugrain TS had a lower ($P < 0.05$) jejunum viscosity versus 0, 50, 75 and 100g (2.41 versus 3.92, 3.82, 2.98 and 2.58 mPa.s). Birds fed 150

g Natugrain TS had lower ($P < 0.05$) 28d necrotic enteritis (NE) lesion score than birds fed 0, 50, 75, 100, and 125 g Natugrain TS (1.87 versus 2.95, 282, 2.41, 2.24 and 2.13 NE lesion score). There was a close correlation ($R^2 = 0.97$) between jejunum viscosity and lesion score decline as Natugrain TS dose increased. Birds fed 125 g Natugrain TS had a higher ($P < 0.05$) IDE versus 0, 50, 75 and 100g Natugrain TS (3177 versus 3106, 3112, 3138 and 3152 kcal/kg). 125 g Natugrain increased ($P < 0.05$) IDE by 71 kcal/kg versus NC. Birds fed 125 g Natugrain TS had a higher ($P < 0.05$) 0–28d broiler weight gain versus 0, 50 and 75g Natugrain TS (1523g versus 1450, 1464 and 1478g). Birds fed 125 g Natugrain TS had a lower ($P < 0.05$) corrected FCR than 0. 50, 75 and 100 g Natugrain TS (1.436 versus 1.478, 1.472 and 1.464). In summary, Natugrain TS decreased ($P < 0.05$) jejunum viscosity, NE lesion scores and FCR, increased ($P < 0.05$) IDE and the optimum bird performance at 28 d was reached with 100 to 125 g/MT Natugrain TS.

Key Words: NSPase, jejunum viscosity, ileal digestibility of energy, necrotic enteritis, clostridium perfringens

375P Effects of the combination of curcumin and polyvinylpyrrolidone with or without boric acid on *Salmonella* Enteritidis colonization and gut health parameters in broilers chickens. J. Latorre*[1], D. Hernandez-Patlan[2], B. Solis-Cruz[2], M. Baxter[1], X. Hernandez-Velasco[3], R. Merino-Guzman[3], B. Hargis[1], R. Lopez-Arellano[2], and G. Tellez-Isaias[1], *[1]University of Arkansas, Fayetteville, AR, [2]Autonomous University of Mexico, Cuautitlan Izcalli, Estado de Mexico, Mexico, [3]Autonomous University of Mexico, Mexico City, Federal District, Mexico.*

Recent restrictions on the use of antibiotics as growth promoters in animal production have pressured the poultry industry to look for alternatives that can continue to provide performance benefits and final products free of food-borne pathogens. Therefore, the objective of the present study was to evaluate the in vitro and in vivo antimicrobial and gut health protective activity of the combination of curcumin (CUR) and polyvinylpyrrolidone (PVP) with or without boric acid (BA). At the beginning of the study, in vitro assays were conducted to evaluate the solubility of CUR with or without PVP at multiple pH values, as well as, its permeability in Caco-2 cells. Results confirmed that the combination of CUR with PVP (CUR/PVP) at a 1:9 ratio significantly increased the bioavailability of CUR. The antimicrobial activity of CUR/PVP with/without BA against *Salmonella* Enteritidis (SE) was determined using an in vitro digestive model that simulated crop, proventriculus, and intestinal compartments. The results revealed that in the proventriculus compartment significant reductions of SE were observed in all the treatments ($P < 0.05$). In the intestinal compartment, 1% BA and the combination of 0.5% BA and 0.5% CUR/PVP (BA-CUR/PVP) showed a synergistic effect on antimicrobial activity against SE. To further evaluate these in vitro findings, 2 independent in vivo trials were conducted to determine the effect of the dietary inclusion of 0.1% BA; 0.1% CUR/PVP or the mixture of 0.05% BA and 0.05% CUR/PVP (BA-CUR/PVP, 1:9) on the prophylactic antimicrobial activity against SE, intestinal permeability and inflammatory responses in broilers chickens. The results showed that BA-CUR/PVP significantly reduced SE colonization in the crop ($P < 0.01$), while CUR/PVP and BA-CUR/PVP showed a significant decrease in SE cecal tonsils colonization compared with control ($P < 0.05$). Administration of CUR/PVP reduced the intestinal permeability of fluorescein isothiocyanate-dextran (FITC-d) when compared with the control non-treated chickens ($P < 0.05$). Additionally, CUR/PVP increased superoxide dismutase (SOD) activity and reduced intestinal IgA levels when compared with the control group. Similar values in performance parameters were observed between treatments. In conclusion, the dietary inclusion of CUR/PVP with or without BA could be a feasible alternative to reduce SE colonization and improve intestinal integrity parameters in broiler chickens. Additional studies will be conducted to confirm and extend these findings using a therapeutic SE challenge model.

Key Words: gut health, *Salmonella*, inflammation, feed additive, broiler

376P Evaluating microbial populations in three sections of the left and right ceca from broiler chickens fed two different diets. M. Baxter*[1], S. H. Park[2], S. A. Kim[3], S. Ricke[1], J. Latorre[1], B. Hargis[1], and G. Tellez-Isaias[1], *[1]University of Arkansas, Fayetteville, AR, [2]Oregon State University, Corvallis, OR, [3]Ewha Womans University, Seoul, Korea.*

The ceca are the primary site of microbial fermentation and water reabsorption in chickens. Digesta fills each cecum through the ileocecal junction at different rates while daily cecal evacuation typically occurs simultaneously. Cecal filling and evacuation depend on contractions, which may suggest that each cecum has a variable microenvironment. Previously, we have shown significant differences between the microbiome of chickens fed with a corn-based (CB) diet versus a rye-based diet (RB). The objective of this project was to evaluate the microbiome in 3 sections of the left and right ceca in broilers consuming 2 different diets. Broiler chicks (n = 5/group) were fed a CB or RB diet. At 10 d of age, chicks were euthanized, and the left (L) and right (R) ceca were collected. Each cecum was further separated into 3 sections: proximal (P), middle (M) and distal (D). Bacterial genomic DNA was extracted from the samples, and the V4 region of 16S rRNA gene was amplified. Amplicons were sequenced on Illumina MiSeq, and microbial communities were analyzed by using QIIME. Results were analyzed using ANOVA, comparing microbial population within and between each cecal pouch within each diet. Out of 6 phyla evaluated, the only significant difference observed was a higher abundance of Actinobacteria in the L/D section when compared with the L/P in the CB chickens ($P < 0.05$). At the family level, out of the 21 families evaluated, *Coriobacteriaceae* in the L/D section was significantly more abundant than the L/P in the CB chickens. Between the right and left ceca in chickens receiving a RB diet, *Peptostreptococcaceae* (P) and Lachnospiraceae (D) were significantly more abundant in the left cecum when compared with the right cecum, while *Lactobacillaceae* (D) was significantly more abundant in the right cecum compared with the left cecum. At the genus level, out of 20 genera evaluated, only *Eggerthella* was significantly more abundant in the L/D section compared with the L/P in chickens fed CB diet. In chicks consuming the RB diet *Oscillospira* was significantly more abundant in the L/P section compared with the R/P. While subtle differences were occasionally observed, these results suggest that there is remarkable uniformity within and between ceca with regard to microbial populations of broiler chickens receiving the same diet.

Key Words: microbiome, ceca, rye, corn, broiler

377P Effect of dietary raw potato starch levels on intestinal barrier function in meat duck from 1 to 35 days of age. S. Qin*, K. Zhang, X. Ding, J. Wang, S. Bai, and Q. Zeng, *Sichuan Agricultural University, Chengdu, Sichuan, China.*

The gastrointestinal tract is covered by a thick layer of mucus that forms the front line of innate host defense. The disruption of mucosal barrier function can lead to increased deposition of toxins and inflammation, and further result in several intestinal disorders. Tight junctions play a crucial role in maintain of epithelial barrier function. Thus, the present

study explored the effect of dietary supplementation with raw potato starch (RPS) on mucosal barrier function in the ileum of duck. 360 male Cherry Valley ducks (8 pens of 15 ducklings on each diet), 1 days of age, were randomly divided into different RPS levels (0% (control),12% and 24%) in diet group for 35d feeding. The mucosal barrier function was investigated by determining ileal morphology, plasma cytokine and endotoxin level, and relative genes expression, and data were analyzed by one-way ANOVA using the Glimmix procedure of SAS 9.2. On day 35 (of age), over 12% RPS supplement in diet resulted in higher weight of ileum, although not significant. Moreover, ducks fed 12% RPS significantly increased villus height and V:C (villous height/crypt depth) compared with other two groups ($P < 0.01$), and consequently to elevate the mucus thickness ($P < 0.001$). Meanwhile, in the 12% and 24% RPS diets, reduced levels of plasma pro-inflammatory cytokine (tumor necrosis factor-α, TNF-α) and endotoxin was observed ($P < 0.05$). Subsequently, supplementation with 12% RPS in diet significantly enhanced tight junction proteins (ZO-1 and Claudin-1) mRNA levels ($P < 0.05$), resulting in improved mucosal integrity. Therefore, results indicate that 12% RPS supplement in diet might be a potential strategy to improve intestinal barrier function for 35-day-old meat ducks through reducing pro-inflammatory cytokine release and improved tight junctions.

Key Words: raw potato starch, intestinal health, mucus barrier function, meat duck

378P Toxic and other heterophil variants of duck blood. P. Cotter*, *Cotter Laboratory, Arlington, MA.*

In avian blood small "resting" lymphocytes out number all other leukocytes. Heterophils exceed both basophils and eosinophils. High numbers indicate inflammation or leukocytosis. The heterophil lymphocytes ratio (H/L) estimates stress. When physiologic demands cause heterophil numbers to increase the H/L will often exceed 0.5; and stress exists. Identification of heterophils relies on recognition of typical cells. However, heterophil morphology may change because of bacteremia or fungemia. Toxic types are recognized by size differences and other peculiarities. Changes of cytoplasmic granules may result in weak staining or atypical granules. The objective is to illustrate a variety of toxic heterophils of duck blood. The method is by a light microscopic examination (Olympus CX-41) of Wright stained venous blood obtained from commercial ducks between ages 4 – 53 wk. Cells were sorted by standard differential counts (SDC, 2 × 200 cells) made at 40x magnification and photographed at 100x (oil) magnification. Cell diameters (D) were measured (μm) using Infinity Analyze Software Release 6.5. Average within sample cell size differences were tested by one-way ANOVA (Minitab Release 17) with significance at $P < 0.05$. The results: size changes include giant heterophils (D >12 μm) and swollen cells (oncosis). Cells displaying an increase in background basophilia; cells with abnormal granulation, cytoplasmic vacuoles; and changes of the nuclear staining intensity (hypo-pigmentation) are also included. Some heterophils display extreme shape distortions in which portions of the mother cell pinch-off into smaller fragments occasionally containing nuclear material. This atypical form named as "Daliosis" is a first description. Discussion: toxic changes of circulating heterophils are believed to result from asynchrony in maturation between the nucleus and cytoplasm due to bone marrow injury from severe inflammation. Giant cells are believed to result from skipped mitotic division(s). Basophilia is believed to result from the retention of protein synthesis machinery (aggregated endoplasmic reticulum) by the cytoplasm. In conclusion, atypical heterophils indicate an abnormal hemogram and complicate the interpretation of H/L ratios. Daliosis involves a fragmentation process rather than cell division. Multiple forms of atypical heterophils may occur in the same sample. The importance of the observations relates to the likeliness of bacteremia/fungemia inferred on cytology and without the requirement for culture. These observations should be important in establishing infection, inflammation and interpreting H/L ratios as stress indicators.

Key Words: atypical hetereophil, size, dwarf, hematology, duck

379P Evaluation of functional feed ingredients as interventions for mitigating necrotic enteritis in experimentally infected broilers. L. Froebel*, E. Kimminau, T. Broderick, and T. Duong, *Texas A&M University, College Station, TX.*

Necrotic enteritis (NE) is a multifactorial disease typically characterized by an over-growth of *Clostridium perfringens* and is estimated to have an annual cost to the poultry industry of 6 billion USD worldwide. Although traditionally managed using antibiotics, consumer preferences and regulatory pressures to reduce their use has increased the need to develop interventions to mitigate effects of NE in poultry production. Functional feed ingredients, those which provide benefits beyond basic nutrition, including dietary prebiotics and botanical extracts may be potentially important alternatives to antibiotics in managing NE because of their antimicrobial effects. In this study, we evaluated the effects of the administration of yeast-derived prebiotic mannanoligosaccharides (MOS) and a botanical extract blend composed of encapsulated cinnamon and oregano essential oils (BOT) to broiler chickens in an experimental co-infection model of necrotic enteritis. Broilers were fed untreated feed or feed supplemented with BMD, MOS, or BOT, and NE was induced experimentally by co-infection with *Eimeria* spp. and *C. perfingens*. Broilers fed untreated feed and mock infected or infected with only *C. perfringens* served as additional controls. Growth performance was evaluated through d 28 post-hatch, while gross intestinal lesions and *C. perfringens* colonization were evaluated on d 21 post-hatch. All data were analyzed using ANOVA. Administration of MOS and BOT improved final body weight ($P = 0.040$) and d 0 – 21 feed conversion ratio ($P < 0.001$) to levels similar to the BMD control when compared with the control infected group. Additionally, administration of MOS improved NE-related mortality and reduced *Clostridium* counts in the jejunum to a level similar to that of broilers experimentally infected with *C. perfringens* alone ($P < 0.001$). These data suggest administration MOS and BOT can may be used to mitigate effects of NE in broiler chickens.

Key Words: necrotic enteritis, functional feed ingredient, botanical extracts, prebiotic, *Clostridium perfringens*

380P Oral endotoxin administration enhances negative effects of heat stress in broilers. U. Hofstetter*[1], B. Grenier[2], N. Reisinger[2], C. Emsenhuber[2], V. Nagl[2], and E. Mayer[2], *[1]Biomin Holding Gmbh, Getzersdorf, Austria, [2]Biomin Research Center, Tulln, Austria.*

The aim of the study was to evaluate the effect of heat stress on gut permeability. Furthermore, it was assessed, if heat stress or oral administration of lipopolysaccharides (LPS) had an effect on the expression of selected genes. Finally, it was evaluated, if the oral LPS administration could enhance the effects of heat stress in broilers. Twenty-eight day old broilers were kept either at 23°C (Thermoneutral) or at 36°C for 10 h. After 7 h of heat stress, half of the birds in each group received LPS orally (2 mg/kg body weight). This resulted in 4 treatment groups with 8 birds per group: 1) Thermoneutral; 2) Thermoneutral + oral LPS; 3) Heat stress; 4) Heat stress + oral LPS. Birds in group 1 and 3 orally received fluorescence labeled (FITC) Dextran (2.2 mg/bird) after 7 h of

heat stress to assess gut permeability. We chose group 1 and 3 only as we could not exclude a reciprocal influence of FITC Dextran and LPS (e.g., binding of LPS to FITC Dextran). At the end of the heat stress period, all birds were euthanized. Blood was sampled to measure the FITC-Dextran concentration in the serum with a fluorescence reader. In addition, intestinal tissue (duodenum, jejunum, ileum) was collected to assess the expression of selected genes via real-time qPCR. For statistical evaluation of data, GraphPad Prism software was used. As data were normally distributed, *t*-test was performed for pairwise comparison of the 4 treatment groups. P-values of <0.05 were considered as significant. FITC-dextran concentration in the serum was significantly increased in heat stressed birds ($P < 0.05$) compared with animals kept under thermoneutral conditions. Heat stress also significantly increased the expression of interleukin 6 in the ileum ($P < 0.05$). When birds were exposed to heat stress combined with oral LPS administration, expression of interleukin 1-β and interleukin 6 was increased significantly ($P < 0.05$) in the duodenum and ileum. The expression of hemeoxygenase was significantly increased in the heat stress group in all parts of the intestine, only when birds were orally challenged with LPS. In conclusion, heat stress seemed to impair the gut barrier function and the expression of inflammatory genes after 10 h of heat stress. Furthermore, the additional oral challenge with LPS enhances the expression of inflammatory genes as well as genes associated with oxidative stress during heat stress.

Key Words: heat stress, endotoxin, LPS, gut permeability, inflammation

381P Identification of *Cochlosoma anatis* in clinically positive and negative commercial turkey samples. K. G. Keen* and R. Beckstead, *North Carolina State University, Raleigh, NC.*

Cochlosoma anatis (*C. anatis*) is a protozoan parasite that has been associated with enteritis, flushing, and decreased body weight gain in turkeys on commercial farms. There are no known treatments or preventative measures for this parasite, resulting in production and welfare concerns. It is unclear how pathogenic *C. anatis* is to the turkey, since most papers report the presence of other pathogens such as hexamita, *Salmonella,* coccidia, or coronovirus in samples positive for *C. anatis*. Understanding the ability of *C. anatis* to cause disease is further complicated since *C. anatis* has not been successfully isolated in culture and there are no experiments studying the individual effects *C. anatis* has on bird health and performance. Variability in the magnitude of field outbreaks suggests that there are compounding factors that affect the pathogenicity of *C. anatis*. It is hypothesized that *C. anatis* can be present in birds showing no clinical signs associated with the parasite until multiple factors initiating *C. anatis* pathogenicity are present. To determine if *C. anatis* is found in healthy commercial turkeys, intestinal samples were obtained from farms both positive and negative for clinical signs associated with *C. anatis*. The samples were screened for the presence of *C. anatis* by polymerase-chain reaction (PCR). Of the 54 samples from clinically positive flocks, 19 (35%) produced a band specific for a portion of the 16S rRNA gene of *C. anatis*. Of the 46 samples from clinically negative flocks, 6 (13%) produced a band specific for *C. anatis*. Not all clinically positive samples were PCR positive, indicating that either samples did not have *C. anatis* or modifications to PCR parameters are needed to raise detection sensitivity of the parasite. Cross-examination of clinically positive and PCR positive birds through a follow-up survey with growers will allow for refinement of PCR as a diagnostic tool for *C. anatis* identification. However, clinically negative samples that were also PCR positive show that *C. anatis* can be found in healthy birds, indicating the potential of an underlying factor contributing to *C. anatis* pathogenicity. Increased sensitivity of PCR diagnostics could lead to a higher percentage of clinically negative samples being PCR positive. This study is the foundation for more in-depth research regarding *C. anatis* to identify factors that contribute to *C. anatis* pathogenicity. Future research will look into differences between clinically positive and negative samples that are also PCR positive through microbiome sequencing.

Key Words: *Cochlosoma anatis*, PCR, turkeys

382P Vitamin D_3 cholecalciferol concentration effects on MQ-NCSU macrophage cell proliferation and macrophage activation. D. Adams*[1], D. Hodgson[1], M. Persia[2], and K. Livingston[1], *[1]North Carolina State University, Selma, NC, [2]Virginia Tech, Blacksburg, VA.*

Vitamin D_3 deficiency is known to have negative effects on the avian immune system. At the same time some are evaluating feeding higher concentrations of cholecalciferol (D_3) to laying hens to enrich their eggs. However, it is unclear as to how these high doses of D_3 will affect macrophage (Mθ) responses. Therefore, this study examined the effect of different levels of D_3 on Mθ proliferation and nitric oxide (NO) production. MQ-NCSU chicken macrophages were used in this study. To determine cell proliferation, MQ-NCSU cells were seeded in 96 well plates with 1.2×10^4 cells/well and exposed to 1 of 4 D_3 treatment (0, 60, 120, 240 ng/ml). D_3 levels were based on previous in vivo study where hens were given various doses of D_3. There were 4 replicate wells per treatment. Plates were removed from incubation and fixed at 24-h intervals for 5 consecutive days before a brdU ELISA assay was performed on each plate. To determine NO production, MQ-NCSU cells were plated in a 24 well tissue culture plate at $1.0x10^6$ cells/well in 1mL of LM HAHN media with the same D_3 levels (0, 60, 120, 240 ng/ml). The plate was incubated for 2 h followed by an addition of 50 ng of lipopolysaccharide from *Salmonella* Typhimurium to each well. The plate incubated for an additional 24 h before supernatants were removed and analyzed via the Griess reagent system, a common system for analyzing NO production. Absorbance was measured at 550 nm. Cell proliferation data were examined using repeated measure ANOVA at 5% significance with SAS 9.4. Macrophage Activation data were examined using Tukey's Test at 5% significance. Cell proliferation analysis showed there were no significant differences in cell proliferation regardless of D_3 treatment. NO production was increased at both the 240 ng/mL and 120 ng/mL level compared with the 0 ng/mL control ($P < 0.05$). Since NO production is used as a direct measure of macrophage activation, it was concluded that the addition of D_3 increases the overall activation of macrophages, and that cell proliferation was not significantly affected by D_3 addition.

Key Words: macrophage, proliferation, nitric oxide, cholecalciferol, cell culture

383P Microbiota population responses to Original XPC™ addition to *in vitro* mixed ceca cultures inoculated with *Campylobacter jejuni*. P. Rubinelli[1], L. Meyer[1], K. Feye*[1], S. H. Park[2], D. McIntyre[3], H. Pavilidis[3], and S. Ricke[1], *[1]University of Arkansas, Fayetteville, AR, [2]Oregon State University, Corvallis, OR, [3]Diamond V, Cedar Rapids, IA.*

Poultry is a major reservoir for the human pathogen *Campylobacter jejuni*. *C. jejuni* inhabits the poultry gastrointestinal tract as a part of the gut microbiota. The goal of this study was to evaluate the population dynamics of the microbiome during an in vitro *Campylobacter* inoculation in the presence or absence of the yeast prebiotic XPC™. Four independent cohorts were conducted. Per cohort, chickens (n = 3) were raised as per standard industry guidelines and sacrificed on D42 post-

hatch. The ceca were aseptically collected and their contents removed and added to 20 mL of sterile Bolton broth in sterile serum bottles. In total, 4 experiments were evaluated: Two experiments (Experiments 1 and 2) contained 0.1% cecal droppings + 1 × 10^8/ml *Campylobacter jejuni* +/ 1% XPC. Experiments 3 and 4 contained 0.1% cecal contents + 1 × 10^8/ml *Campylobacter* +/ 1% XPC. Before the inoculation with the *Campylobacter jejuni*, the cecal contents were pre-incubated with XPC at 42°C for 24h, in a shaking incubator (200 rpm) under microaearobic conditions (5% oxygen, 10% carbon dioxide, 85% nitrogen). Samples of the culture were collected for enumeration on Cefex medium and Petroff counter, and for microbiome analysis, at 0, 24, and 48 h post-inoculation with *Campylobacter*. DNA was isolated using the Qiagen QIAamp Fast Stool DNA Mini Kit. DNA was analyzed using Illumina MiSeq. Reads were filtered, normalized, and assigned taxonomical identities using the MOTHUR pipeline. The relative microbiota populations were compared via 2-tailed *t*-test. Significant reductions ($P < 0.05$) of *Campylobacter* in the presence of XPC were observed by enumeration of the 24h and 48 h time points compared with the 0h time point on both Cefex medium and by counting of *Campylobacter* in a Petroff counter. The same samples upon DNA sequencing of 16s ribosomal DNA V4 region exhibited a significant ($P < 0.05$) increase in *Lactobacillus* only in the XPC-treated cultures. This XPC-dependent *Lactobacillus* increase was observed in all 4 experiments. An increase in Pediococcus was observed in the 1st experiment, but not in the other 3 experiments. We conclude that XPC is capable of significantly reducing *Campylobacter jejuni* in vitro in mixed microaerobic culture, and that this reduction correlated with a significant increase in *Lactobacillus* that was XPC-dependent. Therefore, XPC may potentially contribute to the control in *Campylobacter* when fed to chickens.

Key Words: *Campylobacter*, XPC, microbiota, gastrointestinal tract

384P Blocking intestinal inflammation by aspirin alleviates chicken necrotic enteritis. M. Bansal*, M. Abraha, B. Al-Rubaye, A. Almansour, H. Wang, J. Latorre, B. Hargis, and X. Sun, *University of Arkansas, Fayetteville, AR.*

Necrotic enteritis (NE) in chickens caused by *C. perfringens* and *E. maxima* infection has been responsible for huge economic losses to poultry industry because of reduced usage of prophylactic antimicrobial growth promoters. Birds infected with NE show severe inflammation in small intestine. Cyclooxygenase (COX) signaling is one of the important pro-inflammatory signaling pathways. Here we hypothesized that blocking intestinal inflammation alleviates necrotic enteritis in chickens. To examine this hypothesis, splenocytes isolated from chickens were infected with *C. perfringens* in the presence of 1.2 mM aspirin. Broiler chicks in pen housing were fed diets supplemented with 0 and 0.12 g/kg aspirin. At 18 d of age, birds were orally infected with *Eimeria maxima* (20,000 sporulated oocysts/bird) to induce coccidiosis. The birds were subsequently infected with 10^9 cfu/bird of *C. perfringens* at d 23 and 24 d of age to initiate NE. Growth performance of body weight gain (BWG), feed intake and feed efficiency were measured at d 18, 23, and 26. The birds were sacrificed at 26 d of age. Ileum tissue and content were collected for mRNA expression and histopathology. Histopathology score was based on criteria of villus shortening, immune cells infiltration and crypts hyperplasia. Infecting splenocytes with *C. perfringens* induced significantly higher expression of inflammatory cytokines of IFNγ, LITAF, *IL1β, Mmp9* and *Ptgs2*(COX-2 gene) by 1.54, 1.69, 76.47, 1.72, and 8.65 folds respectively compared with noninfected splenocytes ($P < 0.05$). Interestingly, inhibition of the COX signaling by aspirin attenuated INFγ- or TNFα-induced inflammatory response in the splenocytes. Notably, aspirin diet significantly attenuated NE-induced growth impairment of daily BWG by60%compared with noninfected birds at NE phase of 23–26 d of age ($P < 0.05$). At cellular level, *E. maxima* and subsequent *C. perfringens* infection-induced severe NE showed development of intestinal inflammation. The intestinal inflammation characteristics such as shortening of villi, crypt hyperplasia, and massive infiltration of immune cells was observed in NE infected birds in lamina propria. Consistently, aspirin (0.12 g/kg) in feed significantly attenuated NE-induced histopathological score compared with uninfected (ileitis score 12 vs 16) and villus apoptosis ($P < 0.05$). In conclusion, *C. perfringens* induces inflammatory response in immune cells and chicken intestine, and inhibition of COX signaling attenuates NE. These findings will open new avenue for targeting host inflammatory responses to prevent and treat NE.

Key Words: necrotic enteritis, inflammation, ileitis score, NSAIDs, host inflammatory response

385P The effects of a low-nutrient diet on the prevalence of right ventricle hypertrophy and bursal atrophy in two strains of commercial broilers. D. Rothschild*, T. Widowski, N. Karrow, L. Susta, E. Kiarie, I. Mandell, and S. Torrey, *University of Guelph, Guelph, ON, Canada.*

Conventional broilers are often affected by pulmonary hypertension, right ventricle hypertrophy (RVH), ascites, and immunosuppression, which are all major welfare and production concerns. Previous studies have suggested that reducing growth rate via dietary manipulation can alleviate some of these issues. This study compared the effects of a low-nutrient (LD) diet vs. a conventional (CD) diet on 2 strains of conventional broilers (Strain A and Strain B) to determine if reducing growth through diet can improve cardiovascular health. The LD diet contained 2% lower crude protein throughout the trial. A total of 624 birds from 2 strains of mixed sexed broilers were placed in 24 pens (26 birds/pen; 23.2 kg/m^2). Half of the pens were fed a LD diet, and the other half were fed a CD diet. Feed was provided ad libitum. Biweekly individual body weights (BW) were measured. On d 28, 4 birds/pen (n = 96) were selected as sentinel birds based on BW. Birds were processed on d 46. Carcass weights were determined, along with harvesting hearts and bursa of fabricius. Some were damaged during harvesting, leaving 88 samples. Bursas were weighed and the ratio of bursa:total body weight (B:BW) was determined. Ventricles were removed, separated, and weighed to determine the right ventricle to total ventricle (RV:TV) weight ratio. Data were analyzed using a complete randomized design with a 2 × 2 factorial arrangement of strains (A and B) and diets (LD vs. CD) as fixed effects, using Proc Glimmix in SAS. Overall mortality and culling averaged 1.28%. Diet affected BW at d 42; CD (2.8kg; SEM ± 0.0305) birds were heavier than LD (2.7kg; SEM ± 0.0308) birds ($P = 0.006$). No interactions were observed ($P = 0.47$) between diet and strain on any heart or bursal weights. Diet did not affect RV:TV (LD = 0.155; CD = 0.161; SEM ± 0.0352; $P = 0.11$) or B:BW (LD = 0.131; CD = 0.137; SEM ± 0.0419; $P = 0.48$) between groups. Strain did not affect RV:TV (Strain A = 0.156; Strain B = 0.160; SEM ± 0.0354; $P = 0.62$) or B:BW (Strain A = 0.135; Strain B = 0.133; SEM ± 0.0421; $P = 0.84$) between groups. There were no differences in RV:TV ratio across diet ($P = 0.28$) or strain ($P = 0.52$), although 21 out of 88 (23.9%) birds exhibited below healthy RV:TV ratios based on published literature (0.14 < healthy < 0.24). There were also no differences in B:BW ratio across diet ($P = 0.54$) or strain ($P = 0.78$), although 29 out of 88 (33.0%) birds exhibited lower than healthy B:BW ratio based on published literature (healthy > 0.11). Overall, there were no differences between strains or diets on RVH or bursal atrophy, although a portion of these birds

exhibited signs of impaired cardiovascular health and bursal atrophy when compared with published literature.

Key Words: broiler, diet, right ventricular hypertrophy, bursal atrophy, health

386P Investigation of the relationship between humoral and egg yolk antibodies in laying hens. T. Al-Alwani* and J. Carey, *Texas A&M University, College Station, TX.*

The role of maternally derived antibodies is important to the health of newly hatched chicks. Common vaccination programs attempt to increase the titer of these antibodies in fertile eggs by robust immunization programs for breeder hens. While it is intuitive that there would be a strong relationship between hen humoral titers and egg yolk antibodies, there are no recent data to evaluate the nature of this relationship. The objective of this study was to evaluate the relationship between hen humoral and egg yolk antibody titers. Ten mid- production (40–50 weeks of age) hens were acquired from a commercial poultry company and housed in individual laying hen cages. These hens had previously been subjected to a commercial vaccination schedule in accordance with the health program of the commercial poultry company. This program included multiple vaccinations for Newcastle disease. Hens were fed the TAMU Poultry Research Center standard laying hen diet. Blood samples were collected from the wing vein or jugular vein of each hen 7 times (weekly) throughout the experiment. Blood samples were collected in EDTA tubes and sera were separated by centrifugation. Eggs were sampled from the hens on the day following blood collection. Yolk antibodies for Newcastle disease were purified. Titers to Newcastle disease (NDV) in blood sample and eggs yolk samples were evaluated by ELISA test. Feed consumption and body weight were monitored throughout the experiment. Serum NDV-specific antibody titers were significantly higher than egg yolk antibodies. The correlation coefficient between sera and yolk titers was highly significant with an R^2 over 0.60. In conclusion, this data demonstrates a strong relationship between humoral and egg antibody titers for NDV.

Key Words: antibodies, egg yolk, hen sera

387P Immunofluorescent characterization of the APCs in the chicken bursa of Fabricius using monoclonal anti-CD40 and anti-CD205. J. Skrobarczyk*, C. Vuong, W. Qu, C. Martin, M. Alabdali, E. Caraway, and L. Berghman, *Texas A&M University, College Station, TX.*

The bursa of Fabricius (BF) is best known as a primary immune organ for B-cell proliferation and differentiation that sets birds apart from mammals. However, after the naïve B-cells have migrated to the peripheral immune organs, the BF plays a less recognized role as a secondary immune organ. For instance, at the age of 2 weeks, intra-cloacal immunization with CD40-targeted peptides results in a fast immune response consisting of circulatory IgG and mucosal sIgA production. Throughout, the antigen presenting cells (APCs) of the BF, including the resident macrophages and dendritic cells, play a crucial role in B-cell survival, proliferation and activation. The objective of this study was to improve our understanding of these micro-anatomical relationships through immunofluorescent visualization of the resident APCs within the bursa. APC markers include, but are not limited to, MHC-II, CD40, CD205, and the filamentous protein vimentin. In addition, Bu1 and IgM are recognized B-cell markers. Immunofluorescent detection was performed on tissues from 34-d old birds. CD40, CD205, MHC-II, Bu1, IgM, and vimentin (clone 3B4) were detected using biotinylated and non-biotinylated mouse monoclonal antibodies with fluorophore conjugates. DAPI (4′,6-diamidino-2-phenylindole) was applied as a nuclear stain. Upon examination with a fluorescent microscope, the cortico-medullary border was found to be conspicuously delineated by the expression of CD205, CD40, MHC-II and vimentin. The cortex consisted primarily of MHC-II$^+$ B-cells, while the medulla was populated with B-cells that were strongly positive for Bu1 and surface IgM. Finally, CD40 and CD205 expression was also observed in the cortex and the medulla. These observations were in line with previous reports proposing a dendritic-like cell population lining the cortico-medullary border, whose function is to assist in B-cell survival for transport across the border into the medulla. There, B-cells become fully differentiated and ready for migration to the periphery. The identification of the resident APCs in the BF provided support for the known APC relationships and functions in B-cell development. B-cell proliferation and differentiation cannot be achieved without their assistance.

Key Words: bursa of Fabricius, antigen presenting cell, CD40, CD205, vimentin

388P Assessing a natural feed additive as an AGP alternative for broilers during a necrotic enteritis challenge. A. Çalik*[1], I. Omara[1], N. Evans[2], M. White[1], P. Karnezos[2], and R. Dalloul[1], *[1]Virginia Tech, Blacksburg, VA, [2]PMI Nutiritional Additives, Shoreview, MN.*

This study evaluated the effects of a natural feed additive consisting of a probiotic/prebiotic blend (Bastion) on performance and response of broiler chickens during a naturally occurring necrotic enteritis (NE). On day of hatch, 744 Cobb 500 male broiler chicks were randomly allocated to 3 experimental dietary groups (8 replicate floor pens, 31 birds/pen) as follows: negative control (NC) fed a corn/soybean basal diet; positive control (PC) fed basal diet with Virginiamycin; and additive group fed the basal diet supplemented with Bastion. Upon placement, all birds were challenged by a commercial live oocyst coccidia vaccine applied on the feed and bedding as a predisposing factor to NE. At the onset of NE on d 7, 3 birds from each pen were selected and sacrificed for scoring NE lesions in the inflicted sections of the small intestine per standard protocol. Performance parameters including body weight (BW), body weight gain (BWG), and feed intake (FI) were recorded on d 7, 14, 28, and 42 on per pen basis. Daily mortality and body weight of each dead bird were recorded, and feed conversion ratio (FCR) was corrected by accounting for the BW of each dead bird. Dual Energy X-Ray Absorptiometry (DXA) analysis was performed on 2 birds per pen on d 42 to assess various measurements of carcass body composition. All data were subjected to one-way ANOVA using SAS, significant differences among treatment groups were tested by Tukey multiple range tests and considered significant at $P \leq 0.05$. Dietary supplementation of Bastion significantly improved FCR between d 0–7 ($P = 0.005$), d 15–28 ($P = 0.004$) and d 15–42 ($P = 0.048$). However, no differences were observed among the treatments in terms of BWG, FI, and FCR during the overall experimental period (d 0–42). The anticoccidial treatment (PC) had lower mortality rate when compared with the NC and Bastion. Dietary treatment had no significant effect on NE lesion scores in the duodenum, jejunum, and ileum. Day 42 DXA analysis revealed slight improvements in carcass composition particularly in the form of higher lean content in the Bastion birds compared with the NC controls. These results showed that dietary supplementation of Bastion significantly improved broiler performance during the early NE challenge phase as well as in the grower period.

Key Words: broiler, coccidia, necrotic enteritis, probiotic, prebiotic

389P Effect of feeding lactic acid bacteria isolated from taro skins on growth performance, gut microbiota, and muscle growth of broiler chickens. S. Yadav*, Y. Li, Y. Kim, C. Lee, and R. Jha, *University of Hawaii at Manoa, Honolulu, HI.*

An experiment was conducted to evaluate the effects of 2 probiotic preparations of lactic acid bacteria (LAB) isolated from taro (*Colocasia esculenta*) skins on growth performance, cecal microbiota and muscle growth of broiler chickens. Taro skins samples obtained as by-product from 4 poi manufacturers on Oahu and Maui Islands of Hawaii were collected. After 48 h of natural fermentation of taro skin samples, LAB isolates were cultured on selective MRS agar and *Lactobacillus plantarum* and *Lactococcus lactis* were identified by RAPD-PCR and 16S rDNA gene sequencing. A total of 144 d-old broiler chicks (Cobb 500) were randomly allotted to one of 3 dietary treatments with 8 replicate pens per treatment (6 birds/ replicate pen). The 3 dietary treatments were a corn-soybean meal based diet (control); control plus *Lactobacillus plantarum* (4×10^5 cfu/g feed); and control plus *Lactococcus lactis* (4×10^6 cfu/g feed). Birds were fed for 42 d and feed intake and growth performance data were recoded weekly. On d 42, leg and breast muscle were collected and weighed. Also, cecal digesta samples were collected, cultured and colony count was performed for *Lactobacillus*, *Salmonella*, *Listeria* and *E. coli*. Data were analyzed by ANOVA using Mixed Procedure of SAS, where significant differences among treatments were considered at $P < 0.05$ and means were separated using Tukey's test. Results showed that bird fed with *L. lactis* supplemented diets had significantly lower ($P < 0.05$) ADFI during both starter and finisher period. The BW, ADG, and FCR were not significantly different among treatments ($P > 0.05$). Enumeration of cecal bacteria showed that *L. lactis* fed birds had significantly lower *Listeria* and *E. coli* ($P < 0.05$) compared with control diet fed birds. Relative weights of leg and breast muscles were not different ($P > 0.05$) among the treatments. In conclusion, the supplementation of *L. lactis* isolated from taro skins could be incorporated in the broiler feed to maintain healthy gut ecology by competitive exclusion of harmful bacteria such as *Listeria* and *E. coli.* and promote healthy chicken production.

Key Words: lactic acid bacteria, *Lactobacillus plantarum*, *Lactococcus lactis*, gut microbiota, muscle growth

390P Effect of in ovo injection of probiotic, prebiotic and synbiotic on growth performance and gut health parameters of broiler chickens. L. Li*, A. Singh, B. Mishra, and R. Jha, *University of Hawaii at Manoa, Honolulu, HI.*

Due to the claimed public health concerns, use of antibiotics as growth promoters (AGP) in the chicken feed is banned or regulated in several jurisdictions. Therefore, probiotics, prebiotics, and synbiotics are being evaluated as effective alternatives to AGP to improve growth performance and health of poultry. This study aimed to investigate the effects of *Bacillus coagulans*, Raffinose family oligosaccharides (RFO) and their combination on growth performance and gut health of broilers when injected in ovo. A total of 285 fertilized eggs were divided into 5 groups: i) No-injection group with intact shell, ii) 0.5 mL 0.85% normal saline, iii) Probiotic (*B. coagulans*) (2×10^6 cfu/egg) in 0.5mL 0.85% normal saline, iv) Prebiotic (4.5mg RFO) in 0.5mL 0.85% normal saline, and v) Synbiotic (2×10^6 cfu/egg *B. coagulans* + 4.5mg RFO) in 0.5mL 0.85% normal saline. The injection solution was deposited into the amniotic sac on d 17 of incubation. Hatchability of eggs were recorded. Altogether, 42 d-old chicks from each treatment were randomly allocated to 6 replicate floor pens (n = 8/pen). All birds were raised on a standard commercial diet and management for 42 d. Body weight and feed intake of birds were measured weekly. Ileum samples were collected on d 0 and d 7 post-hatch for RNA isolation. Expression of immune/cytokines related genes in the ileum were determined using qPCR. The in ovo injection did not affect ($P > 0.05$) hatchability of eggs across the treatments. There was no significant effect of treatments on body weight, average daily gain and feed intake of broilers in different experimental groups. However, birds from normal saline treatment had significantly better ($P < 0.05$) feed efficiency and RFO group had the poorest feed efficiency in the first week of post-hatch period. No significant difference ($P > 0.05$) was found on relative organ weight of birds on d 21 and d 42. On hatch day, expression of *IL4* (inducer of T-cells differentiation) was significantly higher ($P < 0.001$) in the ileum of probiotic group. On d 7, immune-related genes (*CD56, ChB6, TLR4, MCN2*) and cytokines-related gene (*IL10*) were significantly higher ($P < 0.05$) in the ileum of saline-treated group, whereas glucose transporter (*SGLT1*) had lower expression ($P < 0.05$) in synbiotic group. In conclusion, in ovo injection of probiotic enhances gut immunity of chicken which would be beneficial for gut health. It is interesting to find that in ovo injection of saline also enhanced gut immunity.

Key Words: gut health, in ovo injection, prebiotic, probiotic, synbiotic

391P Enteric challenge models and compensatory growth in broiler. E. Krabbe*[1], C. Contreira[2], and V. Avila[1], [1]*Embrapa Swine and Poultry, Concórdia, Santa Catarina, Brazil,* [2]*Federal University of Pelotas, Pelotas, Brazil.*

The aim of this study was to evaluate an enteric challenging model as a subsidy for the development of research using additives as alternatives to antibiotics. 1,200-broiler male, Cobb MX, from one to 42 d, were used in a completely randomized block design with 4 treatments and 12 replicates of 25 birds per flor pen. A 2 × 2 factorial design was used. The treatments were: T1 challenged animals that did not receive antibiotics or alternatives in the diet; T2 challenged animals that received antibiotics (Avilamycin 50 ppm) in the diet; T3 animals without challenge and without antibiotic; T4 animals without challenge and with antibiotic (Avilamycin 50 ppm). The challenge consisted of oral coccidiosis vaccine (Bio-Coccivet R) administration on the 6th day, 10-fold the dose recommended by the manufacturer and inoculation with Clostridium perfringens ($2{,}2 \times 10^8$ cfu/g - *Clostridium perfringens* isolated from field in Brazil in 2007) 1 g/bird/day on the 14th, 15th and 16th day, sprayed on the pelleted feed delivered immediately after 5 h fasting (16g/bird/day). Regular feed was returned after 2 h inoculated feed intake. Cumulative Body Weight Gain (BWG) and Feed Conversion ratio (FCR) at 21 and 42 d was measured and submitted to ANOVA (5%), using Statistix software. It was concluded that at 21 d that the challenge model was effective and presented interaction with Antibiotic Growth Promoter (AGP) for BWG ($P < 0.0136$). For FCR there was a significant difference for treatments with and w/o AGP ($P < 0.0019$) or Challenge ($P < 0.0051$), but no interaction was observed. However, at 42d, all effect of treatments tested disappeared. These results indicating that bird could compensate all negative effect of the challenge.

Key Words: additives, antibiotic, body weight gain, feed conversion

392P Tibia density of broilers supplemented with micromineral sources. I. Almeida*, I. Paz, M. Tse, M. Borges, C. Ouros, and G. Chaves, *UNESP/FMVZ, Botucatu, SP, Brazil.*

As sources of organic microminerals should be more bioavailable, so its absorption and use must be more efficient, the aim of this study was to evaluate the effect of zinc sources in tibia density of broilers. For the experiment, were used 1,134 male chicks one day old of the Cobb®500 line, equally distributed in the treatments. The experimental design was a completely randomized with two treatments (T1 - control: 80ppm of Zn, 80ppm of Mn and 10pmm of Cu as inorganic source; T2: 80 ppm of Mn and 10 ppm of Cu as inorganic source + 40 ppm of organic Zn from 1 to 42 days) and nine replicates with 63 birds each. The inorganic Zn source was zinc sulfate and organic source was an amino acid complex. At 43 days, after the slaughter, the tibia of birds was deboned and evaluated for dry matter and Seedor index of bone. The Seedor index was calculated from bone weight and length as [Seedor Index = bone weight (mg) / bone length (mm)]. The data were analyzed to ANOVA and compared by the F test ($P < 0.05$) using the statistical program SAS 9.2. The broilers fed treatment 1 had lower ($P < 0.05$) Seedor index parameter (0.096 value) than animals fed treatment 2 (0.098 value). The higher value of Seedor index demonstrate denser bones, showing that the use of organic micromineral was satisfactory, even at lower concentrations when compared to the use of inorganic zinc. In conclusion, the substitution of this mineral in inorganic form by the organic complex can be successful, resulting in the same parameters of bone quality, with potential for reduction of environmental contamination.

Key Words: bone quality, organic mineral, zinc

393P The effect of exposing layer eggs to light during incubation on early life plasma corticosterone concentrations and on antibody production in response to in-ovo vaccination and vaccination on day of hatch. J. Nelson* and G. Archer, *Texas A&M University, College Station, TX.*

Providing light during incubation has been illustrated to effect hatchability, embryo growth, and post hatch fear and stress. While previously it has been demonstrated that corticosterone concentrations are lower and antibody titers are higher in birds several weeks after hatch in birds exposed to light during incubation the time right after hatch has not been examined. To determine if exposing embryos to light during incubation effected antibody titers and corticosterone immediately following hatch we incubated layer eggs and either exposed them to light or didn't. A subset of each lighting treatment was also vaccinated against New Castle Disease (NDV) in-ovo, or at hatch or not at all. This created 6 treatment groups: Light exposed non-vaccinated (LNV), light exposed in-ovo vaccinated (LIV), light exposed post-hatch vaccinated (LPHV), dark incubated non-vaccinated (DNV), dark incubated in-ovo vaccinated (DIV), and dark incubated post-hatch vaccinated (DPHV). To determine corticosterone concentrations blood samples were collected at hatch, 7 d following hatch, and 14 d after hatch. These blood samples were also used to determine anti-NDV titers. Data was analyzed using GLM: a significant difference was considered $P < 0.05$. The chicks that were incubated with light had lower ($P < 0.05$) plasma corticosterone concentrations at hatch (73.1 ± 21.3 ng/dl), 7 d post-hatch (66.2 ± 20.2 ng/dl), and 14 d post-hatch (62.4 ± 29.4 ng/dl) when compared with the dark incubated chicks (185.1 ± 53.7 ng/dl; 135.9 ± 48.7 ng/dl; 112.1 ± 21.4 ng/dl). The LIV chicks had higher titers ($P < 0.05$) at hatch (3158 ± 259), 7 d post-hatch (4129 ± 351), and 14 d post-hatch (1983 ± 383) than the DIV chicks (2327 ± 320; 3395 ± 365; 1633 ± 384). The LPHV chicks had higher ($P < 0.05$) titers at 7 d post-hatch (3932 ± 355) and 14 d post-hatch (1172 ± 95) than the DPHV (3121 ± 440; 755 ± 198). The LNV and DNV chicks did not differ in titers at any time point ($P > 0.05$). These results demonstrate that exposing layer embryos to light during incubation effects early stress and humoral immunity. This could provide a method to better prepare chicks for the challenges they face at placement.

Key Words: incubation, light, stress, immunity

394P Facility adaptations to trap and remove chicken feathers from manure in the process of sewage disposal. G. Kulkarni*, L. Bacon, A. Fadly, and H. Zhang, *USDA-ARS, East Lansing, MI.*

A method for trapping and removing chicken feathers from manure in the process of sewer disposal for breeders housed at the USDA-Agricultural Research Service Avian Disease and Oncology Laboratory (ADOL) was developed. This piece of adapted and improved feather trap device works efficiently. The feather trap basket was constructed with heavy duty stainless steel to prevent corrosion and deterioration against long-term exposure to acids in chicken manure and high humidity. The trap basket structure consists of a rectangular inverted pyramid frame of 29x 18-inch top and 13x13-inch bottom and 30-inch deep in dimensions. The 4 sides and the bottom of the basket were constructed using stainless steel plates with 0.25-inch staggered pores, which permit remove of manure from the manure-feather mixture by flushing. The 4-side plates were welded to the pyramid frame rods. The bottom plate was constructed to hinge on the bottom frame such that it can be opened, closed, and locked to facilitate feather dumping and trapping function of the trapping basket. A cement concrete pit was constructed under each of chicken cages, which was filled with 2–3 inches of water before manure collection. This permits manure liquidation to ease subsequent flushing and cleaning processes. Each pit was drained weekly into the feather trap basket, followed by flushing and rinsing with water to trap the feathers. A total of 1,914 breeders were housed at 20 weeks of age (1566 hens and 348 roosters) for a period of 24 weeks. A total of 1,587.25 pounds of wet feathers was trapped. This averaged 66.1 pounds per week, during the processes of sewer disposal of the manure. Feather Trapping prevents clogging of sewer drains by a massive quantity of feathers, thereby minimizing costly plumbing repairs. This further ensures farm systems to operate free of or with minimal interruptions, and keeping the disposal relatively more environmentally friendly. The procedures described here may be of interest to other poultry research laboratories and commercial facilities in improving their feather trapping systems.

Key Words: chicken feather, chicken manure, feather trap, environment

395P Effects of preharvest feed withdrawal and aflatoxin in liver in broilers. L. Kindlein*, G. Trentini, F. Bess, and S. Vieira, *UFRGS, Porto Alegre, RS, Brazil.*

Aflatoxins are secondary metabolites of fungi found as contaminants in many food and agricultural products. Effects on broilers include reduced performance, decreased immune function and mobilization of toxic effects. Previous works has shown that the preharvest feed withdrawal causes the hepatic damage in broilers. Therefore, the aim of this study was to evaluate the effect of preharvest feed withdrawal and the intake

of aflatoxin on the color, weight and appearance of the liver of broilers. A total of 42 livers of Cobb chickens, slaughtered at 28 d, were collected. Divided into a control group (n = 21) and a group fed a diet containing 1 ppm of aflatoxin (n = 21) during 28 d, both at 3 preharvest feed withdrawal (12, 6 or 0 h). Weight, macroscopic appearance and color values (lightness- L*, redness-a* and yellowness-b*) were recorded immediately after slaughter. Statistical analysis was performed using the SAS program, GLM procedure ($P \leq 0.001$). Feed withdrawal time resulted in significant linear decreases in liver L*. Livers of control group broilers (0h) were lighter (L* = 33.93, $P < 0.0001$) than treatments subjected to a period for 6 and 12h of feed withdrawal (29.51 and 23.91, respectively), however they had same redness (a*, $P = 0.0808$). Yellowness (b*) was numerically greater (11.80) in livers of without feed withdrawal broilers (0h) than in those from fasted broilers of 6 or 12 h (8.98 and 10.53, respectively), but the differences only were significant between 0h and 6h. These results indicate that as feed withdrawal time increased the surface of the liver appeared darker. The color values measured in the control group (L* = 27.21; b* = 9.60) were lower than in the group that received 1 ppm of aflatoxin (L* = 31.03; b* = 11.28), confirming sensory results obtained in the macroscopic evaluation of hepatic tissues of the group that had the toxin in the diet, such as increase in size, occasional gall bladder distension, pallor and yellowish coloration. It was observed that animals submitted to 12h without food had darkened and pale livers. The liver weight was higher in chickens without fasting (0h), obtaining a loss of 19.03% and 24.52% when compared with animals that had the food removed at 6 and 12 h, respectively. In general, it was possible to observe an increase in the total weight of the liver in animals that received mycotoxin in the diet (2.47%/BW) when compared with the control group (2.20%/BW), suggesting hepatomegaly possibly linked to the accumulation of lipids related to the onset of fatty degeneration developed by hepatic stress. It was observed that the withdrawal of the pre-slaughter food and the intake of aflatoxins have influences on the liver tissue.

Key Words: aflatoxin, toxic hepatitis, fasting, liver color, hepatomegaly

396P Effect of a free-range system on tibia and tendon traits of broiler chickens. E. Martínez-Cruz*[1], J. León-Landeros[1], A. Pro-Martínez[2], A. Vargas-Galicia[2], E. Sosa-Montes[1], B. Bello-Olivera[1], and F. González-Cerón[1], *[1]Chapingo Autonomous University, Texcoco, México, Mexico, [2]Colegio de Postgraduados, Texcoco, México, Mexico.*

Modern broiler chickens reared under intensive system often show leg problems. The objective of this study was to investigate the effect of a free-range raising system on tibia (TiBS) and tendon (TeBS) breaking strength and tibial mineral content (Ca and P) of broiler chickens. One hundred 80 Ross 308 one-d-old straight-run chickens were randomly allocated to 3 treatments: T1, birds raised in confinement and fed ad libitum from 0 to 56 d of age; T2, birds raised in confinement and fed ad libitum from 0 to 21 and from 50 to 56 d of age. From 22 to 49 d of age chickens were grazed 4 h per day in white clover fields and received 50% of the commercial diet consumed by T1 birds; T3, chickens raised in confinement and fed ad libitum from 0 to 21 d of age. From 15 to 56 d of age birds were grazed in white clover fields and received 50% of the commercial diet consumed by T1 birds. All birds were fed with the same starter (0 to 21 d of age) and grower-finisher (22–56 d of age) pelleted diet. Right tibias and calcaneus tendons were collected at 56 d of age (10 birds/treatment) and subjected to breaking strength and mineral content tests. All data were analyzed as a completely randomized design using the methodology of the general linear models. When the model was found to be significant ($P < 0.05$), treatment effects were separated using the Tukey test. Although there were not differences among treatments ($P > 0.05$) in terms of TiBS (126.5 to 144.2 ± 10.2 N) and tibial calcium and phosphorus content (17.1 to 17.6 ± 0.4%, and 4.6 to 4.9 ± 0.1%, respectively), it was observed that birds in T2 and T3 had higher ($P < 0.05$) TeBS (121.4 ± 8.5 and 103.7 ± 8.5 N, respectively) than those in T1 (58.7 ± 8.5 N). On the other hand, TeBS was not different ($P > 0.05$) between T2 and T3. It is concluded that the free-range system improves TeBS of broiler chickens at 56 d of age.

Key Words: tibia, tendon, calcium, phosphorus, broiler

397P Uniformity of Japanese quails submitted to two ages and different types of beak trimming. J. Cruvinel*[1], E. Garcia[1], A. Molino[1], A. Montenegro[1], C. Ouros[2], and K. Alves[1], *[1]São Paulo State University (Unesp)-FMVZ, Botucatu, São Paulo, Brazil, [2]São Paulo State University (Unesp)-FMVZ, Botucatu, São Paulo, Brazil.*

Aim was to evaluate the influence of 2 ages and different types of beak trimming on Japanese quail in uniformity, age at first egg and age at sexual maturity. A total of 630 Japanese quail (*Coturnix coturnix japonica*) were used for the laying phase (36 d - 37 weeks of age), distributed in a completely randomized design, consisting of 7 treatments with 5 replicates of 18 birds each. The treatments were: not trimmed birds (ND), cauterization of about one-third of the beak at 14 d of age (CAUT 14), cauterization of about one-third of the beak at 28 d of age (CAUT 28), moderately beak trimming, removing approximately 1/3 of the beak at 14 d of age (MOD 14), moderately beak trimming, removing approximately 1/3 of the beak at 28 d of age (MOD 28), severe beak trimming, removing approximately 1/3 to 1/2 of the beak at 14 d of age (SEV 14) and severe trimming, removing approximately 1/3 to 1/2 of the beak at 28 d of age (SEV 28). All data were submitted to ANOVA using the resources of the SISVAR program and the means compared by the Tukey test at 5% of probability. Uniformity, age at first egg and age at sexual maturity (50% of production) were not influenced ($P > 0.05$) by treatments. The uniformity was considered very good (80.5%), with a mean of the first egg at 41 d of age and mean sexual maturity at 51 d of age, results that are in agreement with the expected for the species. Therefore, the treatments did not affect the correct growth and development of the quails, much less the beginning of the posture, even in late beak trimming treatments (done at 28 d of age). In view of these results, we can conclude that Japanese quail reached a weight and adequate body structure, allowing the birds to reach uniform and coordinated sexual maturity, thus being able to express all their productive potential.

Key Words: egg production, levels of beak trimming, *Coturnix japonica*, sexual maturity

398P Improvement of radiant heater efficiency by cleaning reflector. H. Li*[1], G. Cartanza[1], and J. Moyle[2], *[1]University of Delaware, Newark, DE, [2]University of Maryland Extension, Salisbury, MD.*

A comfortable thermal environment during growout is critical to the overall performance of a broiler chicken. Heaters are used to create suitable temperatures for growing birds. Two types of supplemental heating methods are commonly used in broiler facilities: radiant tube heaters and forced-air space heaters. Space heaters heat the air in the building, while radiant tube heaters heat the birds and litter directly. Radiant tube heaters do a much better job warming the floor than do forced-air heaters. The reflectors of radiant heat direct heat and energy from the radiant tube, down to the floor. In recent years, many growers have moved away from forced-air heaters because they do not do an

adequate job of directing heat toward the floor and warming the litter. The cleanness of the reflector is critical to deliver as much of the energy from the heated tube to the floor level. The objective of this study was to demonstrate the effectiveness of cleaning reflectors on the efficiency of radiant heaters. Field tests were conducted to compare litter floor surface temperatures in broiler houses before and after cleaning radiant tube heater reflectors. Three radiant tube heaters in 2 broiler houses were tested by using an infrared camera. The reflectors were removed from the radiant heaters and an organic based foaming cleaner was used to clean the reflectors. The results showed that cleaning the reflectors of radiant heaters significantly improved heater efficiency by raising floor surface temperature after cleaning ($P < 0.001$). The average floor surface temperature increased by 2.5 to 5.4 oF. The efficiency of the radiant heater with a dirty reflector decreases when the dirty reflector reflects less heat and absorbs more heat, which leads to higher reflector temperatures and higher ceiling temperatures directly over the heater. To achieve maximum radiant efficiency the underside as well as the top of the reflector typically should be thoroughly cleaned twice a year.

Key Words: radiant, heater, cleaning, reflector, efficiency

399P Effect of a pronutrient (Alquernat Nebsui L) on feed consumption, egg production, egg quality and total carotenoids in laying hens supplemented via drinking water. M. Fulton*[1], C. Domenech[2], T. Dinh[1], A. Kiess[1], and P. Adhikari[1], *[1]Mississippi State University, Mississippi State, MS, [2]Biovet S. A., Tarragona, Spain.*

An experiment was conducted to determine the effects of drinking water application of a phytogenic compound on hen-day egg production (HDEP), egg quality, and egg carotenoids in laying hens. The trial consisted of 2 weeks acclimatization and 9 weeks of testing. A total of 90, 65 weeks-old Hyline W36 hens were randomly allotted into 2 groups, each containing 15 cages (replicates) of 3 hens. The 2 treatments consisted of either with or without a water additive, Alquernat Nebsui L provided via drinking water (0.5 mL/ liter). Hens were housed in conventional cage and fed ad libitum commercial layer feed and water. Egg weight, Haugh unit (HU), yolk%, yolk color score, albumin weight, albumin% were measured. The HDEP, total carotenoids, egg quality data were analyzed using PROC GLM procedures of SAS 9.4. $P < 0.05$ was accepted as statistically significant. There were no significant differences in feed intake between the 2 groups except for wk 4 (94 g vs. 108 g, $P < 0.05$). The average HDEP (%) was significantly higher in the Alquernat group for wk 1, 2, 3, 4, 6 and 9 (all $P < 0.05$). HU was significantly higher for Alquernat group at wk 1, 5, 6 and 8 (all $P < 0.05$). Yolk color index was improved in the hens fed the additive in wk 6, 7 and 8 (all $P < 0.05$). The remaining constraints for internal egg quality were not significantly different between the groups. Eggshell quality was determined by evaluating specific gravity, breaking strength, shell thickness, shell weight and percentage of shell from whole egg weight. The average eggshell breaking strength was similar in both groups for most of the weeks except in wk 1, 5 and 8 where the Alquernat group had a significantly higher breaking strength (all $P < 0.05$). However, no overall differences were observed in specific gravity, shell thickness, shell weight or shell percentage between treatments ($P > 0.05$). Total carotenoids in yolk were significantly improved as hens aged with higher carotenoids in the additive group (all $P < 0.05$). The results of the study suggest that Alquernat Nebsui L may be incorporated via water in laying hens without any negative effects on production and egg quality thus improving total yolk carotenoids. The possible source of yolk pigmentation particularly in later weeks might be due to the ability of the additive that enhanced better absorption resulting in rich yolk color. Further research needs to be carried out on peak-laying age hens as well as the type of carotenoids need to be determined.

Key Words: laying hen, egg quality, carotenoid, hen-day egg production

400P Effects of turkey poult hatch-brood incubation on the gastrointestinal microbiota before and after placement on commercial farms. B. Weber* and T. Johnson, *University of Minnesota, St Paul, MN.*

Standard practice in US commercial turkey production hatches poults in a commercial hatchery followed by transport and placement in a large brood barn where they are raised for 4–6 weeks before movement into a finishing barn. The hatchery itself and the brood environment in which they are placed have major impacts on microbiota development. Companies are exploring hatch-brood systems where poults are hatched and kept in clean and climate-controlled rooms containing platform-housing systems. After 10 d, poults are moved to traditional commercial brood barns. This approach aims to reduce early poult mortality and provide opportunity for optimal microbiota establishment. Our goal was to determine the impact of hatch-brood systems on the developing poult gut microbiota. Day-of-hatch poults were placed either in a hatch-brood system or a conventional brood barn mimicking commercial brood production. After 10 d, hatch-brood birds were moved into the research brood barn separated by fencing but allowing them to share barn microbiota. At d 1, 4, 8, 10, 15, 22, and 29, ceca and ilea content were collected from 10 poults and processed for V4 microbiome sequencing. Microbiome and predicted metagenome analysis was conducted using QIIME and PICRUSt. Taxonomic differences were assessed using DESeq2 with FDR < 0.05 as a cutoff for statistical significance. β-diversity differences were assessed using ANOSIM and α-diversity differences were assessed using nonparametric 2-sample *t*-tests with $P < 0.05$ as the cutoff for significant differences. We identified significant differences in taxonomic abundance between hatch-brood poults and conventionally raised poults. Once combined into a common environment, the microbiota of the hatch-brood poults shifted toward that of the conventional poults and acquired taxa from the conventional barn within days of movement to the barn. β-diversity showed the microbiota from hatch-brood was indistinguishable from conventional brood one week following movement into the same environment. Hatch-brood birds had higher bacterial richness at later time points but higher abundance of microbial markers of performance at early time points. Potential pathogen *Escherichia*/*Shigella* was of lower abundance in the hatch-brood poults when separated, but this increased after introduction into the commercial brood barn. Our results suggest that hatch-brood systems have potential to effectively control the poult microbiota. Further studies are needed to understand the host-microbe dynamics that occur during development within this system and transition into traditional commercial settings.

Key Words: microbiota, turkey, hatch, brood, environment

401P In ovo feeding solution volume and osmolality is critical for optimum hatchability. P. Ferket*, *North Carolina State University, Raleigh, NC.*

In ovo feeding (IOF) provides late term embryos externally-administered nutrients as they imbibe the amnion fluid before pipping. IOF has been demonstrated to improve hatchling quality, advance gut development, enhance development the skeleton, muscles, and immunity, reduce flock weight variability, and improve feed conversion. Hypothesis: hatchability and compatibility with in ovo vaccines may be compromised

by excessive IOF solution osmolality (IOFSO) relative to it's injection volumes (IOFV) when the embryo imbibes the amniotic fluid. Objectives: 1) to develop a mathematical osmotic balance model to estimate maximum IOFSO at different IOFV based on amniotic fluid volume (AFV), embryo serum osmolality (ESO), and amniotic fluid osmolality (AFO) during the period of imbibation; and 2) validate the model estimates by determining the optimum IOFSO administered to turkey embryos at 23 d of incubation (23E) on hatchability and hatch weights of poults. For objective 1, viable eggs from Nicholas turkey hens in 13th wk of lay were distributed among 5 groups of 25 eggs (70–95 g/egg) sampled daily at 1400 h from 20E to 24E to determine AFV, AFO, and ESO so as to calculate max. IOFSO = (ESO(AFV + IOFV) – AFV(AFO))/IOFV. Embryos consumed 4.77 mL AFV from 22E to 23E, and their AFO and ESO was determined to be 248 ± 5.12 mOsm and 310 ± 0.52 mOsm, respectively. Thus, a 1.5mL IOFV was calculated to not exceed IOFSO of 507 mOsm. For objective 2, isocaloric IOF solutions of 253, 542, 863, 1176, and 1455 mOm were prepared using 0.4% NaCl solution containing various levels of dextrin and glucose. At 23E, 75 embryos per treatment groups received 1.5 mL IOF solution returned to standard incubation conditions along with non-injected control eggs until hatch was pulled at 28E. The non-injected controls had 70.7% hatch, but a maximum of 91.7% hatch was observed at IOF of 542 mOsm, and hatchability decreased because of embryonic death before pippin as IOFSO increased further. This study confirmed that excessive IOFSO relative to IOFV (>500 mOsm for 1.5 mL IOF/turkey egg) may cause an osmotic imbalance and embryonic death, and it can be predicted mathematically.

Key Words: in ovo feeding, osmolality, amnion, hatchability, turkeys

402P Effects of infrared beak treatment on the production performance of egg-strain pullets housed in floor pens from 0 to 18 weeks and hens housed in cages from 18 to 60 weeks. S. Struthers* and K. Schwean-Lardner, *University of Saskatchewan, Saskatoon, SK, Canada.*

The objective of this study was to determine the impact of infrared beak treatment (IRBT) on the body weight, feed intake, egg production and quality of Lohmann LSL-Lite (LW) and Lohmann Brown (LB) pullets and hens. The experiment, a 4x2 factorial arrangement of IRBT and bird strain, was performed in a randomized complete block design. IRBT settings (day of hatch) were adjusted to create 4 beak shapes: shovel (SHV) (top beak much shorter than bottom), step (STP) (intermediate differentiation), standard (STAN) (small differentiation), and an untreated sham control (C). Pullets (n = 640) were housed in floor pens (2 reps per IRBT × strain) from 0 to 18 wk of age. Pullet body weight (BW) was collected on a pen basis at 0, 4, 8, 12 and 16 wk. At 18 wk, birds (n = 576) were housed in conventional cages (6 reps per IRBT × strain) from 18 to 60 wk. Hen BW was collected at 18, 42 and 60 wk. Feed intake (FI) and egg quality (EQ) were measured every 4 wk from 22 to 60 wk. Egg production (EP) was recorded 5 d per wk. LB hens were removed from the trial at 42 wk due to cannibalism in the control birds. The effect of IRBT, strain, and their interactions were analyzed using Proc Mixed (SAS 9.4) with Tukey's range test to separate means. Differences were significant when $P \leq 05$. IRBT had an effect on pullet BW at 4 wk with C birds being heavier than STAN birds (0.30 vs. 0.29 kg). There was no effect of IRBT on hen BW. Strain affected pullet BW from 4 to 16 wk, with LB birds being heavier and hen BW at 42 wk, with LB hens were heavier than LW. At 18 wk, an interaction between IRBT and strain was seen for hen BW. LB were heavier than LW hens in all treatments (trts). During the hen period, IRBT and strain did not affect FI or EP. Both IRBT and strain had an effect on unsaleable eggs. Control hens laid a higher percentage of unsaleable eggs than hens in the SHV and STP trts (1.33 vs. 0.74 vs. 0.7%). LB hens laid a higher percentage of unsaleable eggs than LW hens (1.21 vs. 0.77%). Interactions between IRBT and strain were seen for percentage of broken and abnormal eggs laid. LB hens in the STAN trt laid more broken eggs as compared with LB hens in the SHV and STP trts and LW hens in all trts. LB hens in the STAN trt laid more abnormal eggs as compared with LW hens in the SHV, STP, and STAN trts. There was no effect of IRBT on EQ. Strain affected EQ with LW hens producing heavier eggs than LB hens (60.7 vs. 58.1 g) but LB hens having better specific gravity than LW hens (1.088 vs. 1.086). Overall, the results indicate that IRBT may reduce early BW, but does not negatively impact the production performance of LW and LB pullets and hens and that strain may play a role in BW, FI, and EP.

Key Words: Lohmann, unsaleable eggs, shovel beak, body weight

403P Effect of a free-range raising system on leg soundness traits of broiler chickens. J. Orzuna-Orzuna*[1], J. León-Landeros[1], E. Martínez-Cruz[1], A. Pro-Martínez[2], A. Vargas-Galicia[2], E. Sosa-Montes[1], and F. González-Cerón[1], *[1]Chapingo Autonomous University, Chapingo, Mexico, Mexico, [2]Colegio de Postgraduados, Campus Montecillo, Montecillo, Mexico, Mexico.*

An intensive raising system combined with the high growth rate of the modern broiler chicken often lead to leg problems and reduced productive performance. The present study was carried out to investigate the effect of a free-range raising system on walking ability [gait score (GS)], valgus/varus angulation (VAng) and latency to lie (LTL) of broiler chickens. Six score categories (0 to 5) were used for GS evaluation, where 0 is a bird with fluid locomotion and a furled paw when it is raised, and 5 is a bird in complete lameness that cannot walk or stand. VAng was evaluated according to a scale from 0 to 3, in which: 0, normal chicken, and 3, severe angulation (angle greater than 45°). A total of 180 one-day-old mixed chickens (Ross 308) were randomly allocated to one of 3 treatments: T1 or control, birds raised in confinement and fed ad libitum from 0 to 56 d of age; T2, birds raised in confinement and fed ad libitum from 0 to 21 and from 50 to 56 d of age. From 22 to 49 d of age chickens were grazed in white clover fields (4 h/d) and received 50% of the commercial diet consumed by T1 birds; T3: chickens raised in confinement and fed ad libitum from 0 to 21 d of age. From15 to 56 d of age birds were grazed in white clover fields and received 50% of the commercial diet consumed by T1 birds. All birds were fed with the same starter (0 to 21 d of age) and grower-finisher (22–56 d of age) pelleted diet. Twenty birds per treatment (10 male and 10 female) were randomly selected to assess LTL at 49 d of age, and VAng and GS at 49 and 56 d of age. All data were analyzed as a completely randomized design. However, for GS and VAng data the methodology of the generalized linear models was used. A frequency analysis was also perfomed for GS and VAng. In contrast, LTL data were analyzed using a general linear model. Two fixed factors were considered in all models: treatment and sex of the bird. There was no effect of treatment, sex or its interaction on LTL ($P > 0.05$). GS and VAng were not affected by neither treatment nor sex or the interaction at 49 d of age ($P > 0.05$). However, at 56 d of age, raising system affected ($P < 0.001$) GS and VAng. While 95% of birds in T1 had GS equal or higher to 2, none of the chickens in T2 and T3 showed similar GS. Likewise, 86% of chickens in T1 had VAng scores equal or higher to 2. In contrast only 8% of birds in T2 had similar scores, and none of the chickens in T3 showed those VAng values. In conclusion, a free-range raising system improves leg soundness in terms of GS and VAng in broilers at 56 d of age.

Key Words: leg soundness, free-range, broiler

404P The impact of low-nutrient and conventional diets on lameness, tibial dimensions and tibial ash concentrations for two fast-growing genotypes of broiler chickens. M. Nascimento dos Santos*, T. Widowski, E. Kiarie, M. Mohammadigheisar, I. Mandell, and S. Torrey, *University of Guelph, Guelph, ON, Canada.*

Lameness is a critical welfare issue for the broiler industry, with fast growth being a causal factor. Diets may play an important role in both growth rates and lameness. Therefore, this study evaluated the impact of 2 diets (low-nutrient and conventional diet) on lameness, tibial dimensions, weight, ash concentration, and body weight (BW) in 2 commercial lines of broiler chickens. A total of 624 mixed sex birds from 2 fast-growing strains (A and B) of broiler chickens were placed in 24 pens (26 birds/pen; 23.23 kg bird/m^2) and fed either a conventional (CV) or a low nutrient (LN) diet (n = 156). The LN diet had approximately 2% lower CP level compared with the CV diet throughout the trial. From each pen, 12 birds were wing-banded and followed from hatch. Individual BW was determined biweekly. On d 28, 4 wing-banded birds/pen (sentinel birds, n = 96) were chosen based on BW. On d 42, a latency to lie test (a validated method to assess lameness in broiler chickens, with lameness being associated with a reduced latency to lie in shallow water) was performed on sentinel birds. Birds were slaughtered on d 46 (BW = 3.22 ± 0.076 kg) and both right and left tibia from sentinel birds were removed to determine tibial length, diameter, and tibial fresh weight (total and relative to BW). Bone ash concentration (%) was measured in the left tibia to estimate bone mineralization and composition. Data were analyzed as a complete randomized design with a 2 × 2 factorial arrangement with strains (A and B) and diets (LN and CV) as main factors and with final BW as a covariate, using Proc Glimmix in SAS. No interactions between diet and strain were observed for any parameter evaluated ($P > 0.05$). There was an effect of strain on BW at d 14 ($P < 0.0001$), d 28 ($P < 0.0001$) and d 42 ($P < 0.0001$), with heavier BW observed in strain A compared with strain B (248 g difference at d 42). Diet only affected BW at d 42 ($P = 0.01$). Birds fed CV diet were 119 g heavier than birds fed the LN diet. Latency to lie was not affected by diet ($P = 0.84$) or strain ($P = 0.40$). Tibial length was not affected by strain ($P = 0.08$) or diet ($P = 0.09$). However, birds fed the CV diet had a greater tibia diameter compared with the LN diet ($P = 0.04$). Consistently, tibial fresh weight ($P = 0.02$) and tibial fresh weight:BW ratio ($P = 0.01$) were greater for the CV-fed group. Tibial ash concentrations were not affected by strain ($P = 0.43$) or diet ($P = 0.52$). Strain influenced BW, but did not affect tibial morphology or lameness. Diet density affected BW at d 42, tibial diameter and weight, but not lameness. Further studies should investigate if slower growing strains of broilers have improved leg health and welfare.

Key Words: diet, strain, broiler, growth, leg health

405P The effect of Activo Liquid essential oil intake during the pullet to layer transition period on early egg production and egg quality in Lohmann LSL-Lite. M. Temple*[1], T. T. O'Lear Reid[1], J. Walls[2], R. Cabrera[2], and D. Bennett[1], *[1]California Polytechnic State University, San Luis Obispo, CA, [2]EW Nutrition USA Inc., Des Moines, IA.*

The transition from pullet rearing stage to full production is a stressful and demanding period in the life of a laying hen. Previous studies demonstrate that plant extracts and essential oils have a positive effect on production performance and health of laying hens, but few studies have examined the use of these products during the pullet to layer transition period. Our objective was to evaluate the use of Activo Liquid (EW Nutrition), a commercially available blend of essential oils, on the livability of pullets during the pullet to layer transition, and on the development of their subsequent egg production. Lohman LSL-Lite pullets (1,380 total) were reared in conventional cages and transferred to 64 enriched layer cages at 18 weeks of age. Birds received Activo Liquid in their drinking water (0.366 mL Activo/L) for 0, 3, 5 or 7 d, either pre- or post-transfer to the laying facility in a 4x4 factorial design, with 4 replicate cages per cell. Mortality was monitored throughout the experiment. Egg production was recorded daily between 19 and 26 weeks of age, and again at 35 weeks of age. At 25 and 35 weeks of age, all eggs were collected, weighed, and assessed for eggshell cracks (checks). Two eggs per cage were randomly selected to assess egg shell quality (weight, thickness, and breaking strength). Data were analyzed with repeated measures ANOVA, with the length of time birds were provided with Activo Pre- and Post-transfer as main effects and week of measure (age) as the repeated effect. Post-hoc LSD means separation were performed to separate treatment effects. No mortality was recorded for hens between 18 to 26 weeks of age. Pre-transfer dosing of Activo significantly affected egg production between 19 and 24 weeks of age, attributed to lower egg production in the 7-day treatment group. Pre-transfer dosing of Activo did not significantly affect egg production or eggshell quality at 25-26 and 35 weeks of age. Post-transfer dosing of Activo did not significantly affected egg production between 19 and 24 weeks of age. However, egg production was significantly affect at 25-26 weeks of age, associated with higher production in the 3-day treatment group, but not at 35 weeks of age. Post-transfer dosing of Activo did not significantly affect eggshell quality. Based on results collected during this study, we recommend 3-day post-transfer dosing of Activo Liquid to improve early commercial layer production.

Key Words: essential oils, egg production, eqq quality, laying hens

406P Supplementation of extruded red seaweed (*Chondrus crispus*) in laying hen production. C. Stupart*, J. MacIsaac, and B. Rathgeber, *Dalhousie University, Truro, NS, Canada.*

Inclusion of feed ingredients for the purpose of modifying the bacteria in digestive tract of poultry has become a concept of interest in effort to reduce the use of antibiotics. Previous studies have indicated that the red seaweed *Chondrus crispus* (CC) provides a prebiotic benefit in short-term laying hen studies. The purpose of this study was to monitor production parameters of late cycle laying hens when the seaweed was processed with an extruder to ensure performance is not compromised. The experiment was a 2 × 4 × 2 factorial in a completely randomized design with processing method for the CC (ground and extruded), inclusion level of the CC (0, 0.5, 1.75, 3%) and strain of hen (Lohmann Lite-LSL White and Lohmann Lite Brown) as the main effects. A total of 400 birds were used in the study, with 4 replicate cages per treatment combination (5 birds per cage). Feed consumption was recorded and averaged over a 28-d cycle. Body weights were measured and recorded at the beginning of each 28-d cycle. The results were analyzed as a factorial arrangement using the Proc Mixed Procedure of ANOVA. Feed consumption was not affected by the level of CC, regardless of processing method. Body weight gains were not affected ($P > 0.05$) by processing method or strain of hen. There was an interaction ($P < 0.05$) between inclusion level of CC and period of production for weight gain. For Period 1, hens fed diets containing 3% CC gained less ($P < 0.05$) weight than those fed the diets containing 0 and 0.5% CC. However, for Period 2, weight gains were similar ($P > 0.05$) for all inclusion levels. Thus, dietary inclusion of CC may serve as a beneficial feed additive at levels of 0.5 and 1.75% without negatively affecting weight gain.

Key Words: red seaweed, prebiotic, laying hen, alternative to antibiotic, gut enhancer

407P Effect of in ovo lighting and egg sanitation on breast muscle development in broilers at hatch. J. Henry*, B. Rathgeber, X. Li, and E. Conners, *Dalhousie University, Truro, NS, Canada.*

Incubation has become an increasingly important period of chicken production, as the time it takes to rear a market sized chicken continues to decrease. Past research has reported carcass part yield differences in birds incubated under different lighting programs. In the current study the role of photoperiod during incubation on breast muscle development was investigated. Light was provided during incubation of 144 Cobb 500 hatching eggs. These eggs were sanitized with a 3% lysozyme solution or water for 10 min before incubation and at the time of transfer to the hatcher. The treatment combinations were randomly assigned to 8 Chick Master G30 incubators. Half of the incubators were equipped with dim to red LEDs while the other 4 were kept in complete darkness. Incubators with red lighting were on a lighting photoperiod of 12 h of light per day. On day of hatch, 2 chicks per sex per treatment (n = 32) were euthanized and dissected to determine yolk free body weight and breast muscle weight. When performing breast muscle removal, muscle tissue with keel intact was removed and weighed. An ANOVA using proc mixed procedure was performed for statistical analysis, with a p-value < 0.05 being considered significant. The yolk free body weight and breast muscle mass were not different among treatments ($P > 0.05$). The use of light with this type of spectrum did not influence muscle growth as measured in this study.

Key Words: incubation photoperiod, egg sanitation, lysozyme, breast muscle, chick weight

408P Effect of using cinnamon and ginger powder as feed supplement on performance and carcass quality of broiler. Dhananjay Gaikwad*[1] and Y. Fulpagare[2], *[1]Lovely Professional University, Punjab (India), Phagwara, Punjab, India, [2]PGI, Mahatma Phule Agriculture University, Rahuri, Maharashtra, India.*

The effect of feeding broiler chicks on diet containing different levels of cinnamon and ginger powderas natural feed supplement on growth performance, and sensory evaluation were studied. A total of 140, day old 'Vencob-400' chicks, which were divided into 7 treatment groups (Five chicks per replicate) i.e., 20 chicks per treatment groups. Dietary treatments consisted of basal diet with no additives T_0(Control), T_1, T_2, T_3, T_4, $T_5$and T_6receiving 1.0%, 2.0% and 3.0% cinnamon and 1.0%, 2.0% and 3.0% ginger, respectively. Cinnamon and ginger feeding were done separately and compared by completely randomized design (CRD). The body weight of the 2.0% (T_2) cinnamon and 1% ginger (T_4) group was significantly ($P \leq 0.05$) higher than the other groups. The average feed consumption vary significantly ($P \leq 0.05$) from control group. The average feed consumption was highest (3966.85 g/b) in control group and lowest (3793.30 ± 05.94 g/b) in 2.0% cinnamon (T2) supplemented group. No mortality was observed either in control (un-supplemented) or cinnamon and ginger supplemented groups. In addition to, the inclusion of cinnamon and ginger powder in broilers diet showed significantly ($P > 0.05$) higher flavor scores. From the result of present study it could be suggested that dietary inclusion of 2.0% cinnamon and 1.0% ginger can be used as growth promoters and improve carcass quality.

Key Words: growth, carcass, quality, broiler, production

409P Effects of dietary amino acid reduction on the meat yield of six strains of broilers. E. H. Lovelace*, B. Zhang, and Wei Zhai, *Mississippi State University, Mississippi State, MS.*

The effects of limiting the amount of amino acids in diets were determined on 6 strains of broilers. Eggs from breeder hens of the 6 strains with similar age (30 wk of age) were hatched together in the same incubator. A total of 1,504 d-old hatchlings were randomly placed among 8 blocks in an environment controlled broiler house. Each of the 8 blocks received all the 12 treatments. A total of 16 broilers were placed in each experimental unit pen. The layout of the treatments was 6 (strains) × 2 (diets) factorial arrangement. Of the 6 strains of broilers there were 5 modern commercial strains and a control meat-type strain developed in 1955, Athens Canadian Random Bred (ACRB). The 2 diets that were given to the broilers consisted of one diet with normal levels of amino acids (AA) (meet or exceed the nutrient requirement of all commercial broilers according to primary breeder recommendations) and one diet of 20% reduced amino acids (digestible lysine, total sulfur AA, and threonine). Four birds per pen were randomly selected, weighed, mechanically processed, and manually deboned on d 42 and 56. Parts were weighed and relative weights of parts to BW and carcasses were calculated. Data were analyzed using 2-way ANOVA using PROC GLM of SAS 9.4. On d 42, carcass weights were decreased due to dietary AA reduction in all 5 modern commercial broiler strains ($P = 0.007$). On d 56, carcass weights were decreased in only 3 modern commercial broiler strains due to AA reduction ($P = 0.001$). In a similar manner, breast weights were reduced in birds fed AA reduced diets in all commercial broiler strains on both d 42 and 56 ($P < 0.0001$, $P = 0.0002$). Relative breast weights to BW in most commercial strains were still higher in birds fed normal AA on d 42 and 56 ($P = 0.041$, $P = 0.035$), with exceptions of 2 strains which exhibited similar relative breast weights to BW on d 42. In opposition, abdominal fat pad weight increased in the AA reduced groups on both d 42 and 56 ($P < 0.0001$, $P = 0.016$), which may be due to an imbalance of AA and energy composition of the diets. One of the commercial strains exhibited lower fat pad weights as compared with the other 4 strains on both d 42 and 56 ($P = 0.003$, $P = 0.0002$). However, the carcass or breast weights of ACRB were not affected by AA reduction on d 42 or 56. No abdominal fat pad was observed in ACRB birds. In conclusion, AA reduction affected meat yields of various broiler strains at various ages differently. In addition, different parts of the body responded to AA reduction differently. Breast growth of modern commercial broiler strains was more sensitive to AA reduction than the rest of the body, which may due to genetic selection of high breast yield of broilers.

Key Words: amino acid, broiler, meat yield, strain

410P Evaluation of amino acid density on growth performance in 34 day old broilers. J. Leopold*[1], D. Portillo[1], R. Brister[2], R. Latham[2], and J. Lee[1], *[1]Texas A&M University, College Station, TX, [2]Tyson Foods Inc., Springdale, AR.*

In a 3-phase, 34 d-old, small-bird program, the growth performance of Cobb 500 × M99 mixed-sex broilers were evaluated after altering the dietary AA densities in the last 2 feeding phases. The experimental design consisted of 4 dietary treatments (Trts) that were allotted in a randomized, complete block design, with 15 replicate pens/ trt and 50 birds/ replicate pen. A common starter was fed for 12 d; however, in the grower (fed 13–25 d-of-age) and finisher (fed 27–34 d-of-age) phases, 4 dAA densities was fed; all Trts within a phase were formulated to be iso-caloric and dEAA: dLys ratios were maintained across all diets within a given phase. In the grower and finisher phases, the respective dLys values in the lowest AA density diet were 0.95% and 0.82%; representing Trt 1. In the grower and finisher phases, the following adjustments were made against the dLys in the respective control feeds: +0.05% (Trt 2), +0.10% (Trt 3), and +0.15 (Trt 4). These diets were fed throughout the Grower and Finisher phases. All data were subjected to a one-way ANOVA, with means deemed significant at $P \leq 0.05$; significant means were separated using a Duncan's Multiple Range Test. Body weight was increased ($P < 0.001$) with each increased in dLys on d 25 and 34 of age with the difference between the lowest and highest AA density being 111 and 178 g/bird, respectively. The reduction in AA density negatively impacted mortality-adjusted feed conversion ratio (FCR; $P < 0.001$). The incremental increase in dAA density among the 4 treatments resulted in significant, stepwise increases in dLys intake and stepwise reductions in FCR with the difference in d 1 to 34 FCR between the highest and lowest dAA diets being 0.13 (1.629 vs 1.499). Linear regression analysis confirms a positive impact on FCR and body weight with increasing dLys intake demonstrating the importance of dLys intake on broiler growth performance.

Key Words: amino acid density, broiler, performance

411P Supplementation of different levels of choline and digestible total sulfur amino acid for broiler chickens. G. Santiago*, C. Simões, P. Vivan, T. Santos, L. Kindlein, and S. Vieira, *UFRGS, Porto Alegre, RS, Brazil.*

An experiment was conducted to evaluate the growth performance (GP), the occurrence of leg deviations and the fatty liver of broilers fed a corn and soy protein isolate diets supplemented with choline chloride (minimum of 52.2% of choline) and digestible total sulfur amino acid (TSAA). In addition, the maximum response of choline (Cho) supplementation was estimated. A total of 525 one-day-old male chicks were distributed in 15 treatments with 5 replications in a completely randomized design, using 75 battery cages with 7 birds each. A 74% corn semi-purified basal diet (727 ppm of Cho) was supplemented using a 3 × 5 factorial arrangement with 3 levels of digestible TSSA ratio to digestible Lysine (70, 75, 80%); and 5 levels of Cho (0; 700; 1,400; 2,100; 2,800 ppm). Levels of total Cho (supplemented + basal diet) were 727; 1,427; 2,127; 2,827 and 3,527, respectively. A 2-phases (pre-starter and starter) feeding program was used and GP was evaluated until 21 d. At 21 d all birds were evaluated for occurrence of valgus, varus and tibial rotation deviations, tibia width, length and ash content, also livers were evaluated (size, color, fat content). Data were analyzed using the GLM procedures of SAS and the significative differences by Tukey test at 5%. Performance data were fitted to linear and quadratic polynomial regressions and the maximum response of Cho supplementation was estimated. No interactions between digestible TSAA and choline levels, also no differences among digestible TSAA levels were observed from GP. The body weight gain (BWG) of birds fed diets with increasing levels of Cho increased quadratically from 1 to 7, 8–14, 1–14, 15–21, and 1–21 d, also feed conversion ratio (FCR) decreased quadratically from 1 to 14 and 1–21 d. From 1 to 7, 8–14, and 15–21 d quadratic regression estimated requirements as 2,700; 2,907; and 3,105 ppm for BWG. From 1 to 14 and 1–21 d, quadratic regression estimates were 2,875 and 2,925 ppm for BWG; and 2,938 and 2,849 ppm for FCR. Birds fed no supplementation of Cho from 1 to 7, 8–14, 1–14, 15–21, and 1–21 d showed lowest feed intake. Treatments with no supplementation of Cho

had higher varus and rotated tibia deviations. Birds fed 70% of TSAA presented higher liver fat content ($P < 0.10$) than ones fed with 80% at 21 d. In conclusion, increasing levels of Cho presented higher BWG and lowest FCR, also there was lowest varus and rotated tibia deviations for broilers at 21 d. Considering a corn-soybean meal common diet (1,500 ppm of choline), 1,425 and 1,349 ppm of Cho inclusion are appropriate to improve BWG and FCR response for broilers from 1 to 21 d.

Key Words: choline, broiler, total sulfur amino acid, requirement, performance

412P Production performance responses to increasing isoleucine supplementation in a low crude protein diet fed to pullets from 19 to 48 weeks of age. I. Parenteau*[1], M. Stevenson[2], and E. Kiarie[1], *[1]University of Guelph, Port Perry, ON, Canada, [2]Halchemix Canada Inc., Port Perry, ON, Canada.*

Low CP (LCP) diets fortified with synthetic AA are commonly fed to lower feed costs, gastrointestinal disorders and N emissions. Isoleucine is speculated to be potentially limiting in LCP corn-soybean diets fortified with Met, Lys, Trp, Thr and Val; however, supporting data is limited. Therefore, the objective of the current study was to investigate the optimal Ile level in diets containing 2 percentage units lower CP vs. standard (HCP) breeder diet. One hundred and 80 Shaver White pullets were randomly allocated to one of 5 diets (n = 6) in a 2 phase (19–27 and 28–48 weeks of age) feeding program. Isocaloric basal LCP diets were formulated on ideal protein concept with SID Lys concentrations of 0.75 (phase 1) and 0.71 (phase 2), and contained graded levels of synthetic Ile to produce SID Ile:Lys ratios of 0.7, 0.8. 0.9 and 1.0. A standard CP (18 and 16% CP for phases 1 and 2, respectively) diet with an SID Ile:Lys of 0.8 (no added Ile) was also tested. Pullets were housed in cages (6 hens/cage) and had free access to feed and water. Hen day egg production (HDEP), egg mass (EM), feed conversion (FCR), and egg quality (yolk to albumin ratio, Haugh unit) were measured. Data was analyzed using ANOVA in JMP 13.2.1 with diet and phase as fixed effects. Treatment means were separated using multiple comparisons, and effects of Ile supplementation to LCP was evaluated using orthogonal contrasts. Crude protein reduction did not affect feed intake; however, hens fed high levels of Ile (≥0.9 SID Ile:Lys) consumed less ($P = 0.002$). Reduced CP intake decreased HDEP by 2.8% ($P = 0.021$), however EM and FCR were not affected. Yolk to albumin ratio was increased by 4.6% ($P = 0.039$) with a 2% reduction of dietary CP, but was linearly reversed ($P = 0.001$) with the addition of Ile; this was accompanied with a linear increase ($P = 0.002$) in Haugh unit from 68.8 to 72.7. Hens fed 0.9 SID Ile:Lys diet produced the largest eggs overall ($P < 0.0001$), had similar ($P = 0.049$) production to the HCP diet (95.4 vs 96.1%), and had the best FCR ratio of 1.91 in phase 2. Excess Ile (1.0 SID Ile:Lys) resulted in decreased egg weight in both phases and lower HDEP and EM in phase 2 ($P < 0.0001$), resulting in an increased FCR value of 2.05. Overall, this data suggests that supplementing LCP corn-soybean diets with synthetic Ile between the SID level of 0.7 and 0.9 Ile:Lys is comparable to a standard HCP diet in terms of production performance.

Key Words: isoleucine, low crude protein, HDEP, egg weight, egg composition

413P Dry-extrusion of Asian carp to supplement natural methionine in organic poultry production. I. Upadhyaya*[1], K. Arsi[1], B. R. Wagle[1], S. Shrestha[1], A. Upadhyay[1], A. Donoghue[4], C. Coon[1], M. Schlumbohm[1], J. Trushenski[5], C. Owens-Hanning[1], M. Riaz[2], M. Farnell[2], A. Davis[6], A. Fanatico[3], D. Donoghue[1], *[1]University of Arkansas, Fayetteville, AR, [2]Texas A&M University, College Station, TX, [3]Appalachian State University, Boone, NC, [4]USDA-ARS, Fayetteville, AR, [5]Evaqua Farms, Filer, ID, [6]University of Georgia, Atlanta, GA.*

Methionine, a sulfur containing amino acid, is essential for healthy poultry production. Synthetic methionine is commonly used as a supplement in conventional poultry and is also temporarily allowed in organic poultry production, for lack of natural, cost effective sources of methionine. Hence, there is a pressing need to explore alternatives that are a safe source of methionine for organic poultry and egg production. Invasive Asian carp (AC) are a potential natural methionine source, but AC fish meal is not currently available because industrial-scale rendering requires a significant investment in infrastructure and may create nuisance odors. In this study, extrusion technology as a potential cost-effective alternative to rendering was explored. Dry extrusion of AC was carried out by mixing the fish with soybean meal (SBM) in a 1:1 proportion to reduce high moisture in the starting material. Proximate analysis, amino acid composition, digestible amino acids and true metabolizable energy (TMEn) for the extruded AC fish meal were determined. To study the digestible amino acids and TMEn of the extruded product, a colony of adult roosters were fasted and tube fed AC fish meal. The gross energy and protein content of the feces from AC fish meal were determined to calculate the digestibility of amino acids and TMEn for feed formulation. Diets with AC fish meal were formulated and fed to broilers (2 treatments, AC fish meal or Control diet with synthetic methionine; 8 replicates/treatment; n = 30 birds/replicate) from d 1 to 42 d of age. The inclusion level of AC fish meal was 50.13% in AC fishmeal starter and 43.66% in AC fishmeal grower diet. The levels of other ingredients were formulated using BRILL 2.5 software as isocaloric diets to meet broiler nutrient requirements between the treatments. At the end of the trial, data for body weights, feed intake and feed conversion ratio (FCR) was analyzed using one-way ANOVA with Fisher LSD test for multiple comparisons. Results revealed that birds on AC diet had body weight gains and feed intake comparable to diets containing synthetic methionine ($P > 0.05$). Moreover, the FCR for control birds was 1.58 when compared with birds on AC fishmeal, which had an FCR of 1.53 from d 1–42 ($P > 0.05$). Results from the study suggest that invasive AC-derived fish meal could potentially be an effective and inexpensive source of sustainable natural methionine for organic poultry farmers. Future research will focus on sensory and meat quality attributes from birds on AC fishmeal. This study was funded by USDA-NIFA-2015–51106–23966.

Key Words: Asian carp fishmeal, methionine, dry-extrusion, broiler, production parameter

414P Isoleucine requirements of male broilers. N. Sakomura*, M. Melare, M. Reis, and G. Viana, *Universidade Estadual Paulista-UNESP, Jaboticabal, São Paulo (SP), Brazil.*

Broiler requirements for essential amino acids such as lysine, total sulfur amino acids, threonine and valine have been widely studied over the last decades. Isoleucine (Ile) has been reported as the fifth limiting amino acid for broilers fed corn-soybean meal-based diets. The establishment of Ile requirement could be an important step forward reducing dietary crude protein supply for broilers. Three dose-response assays were conducted to estimate male broiler chicken requirements for Ile (Ross 308) in the starter (1–14d), grower (15–28d), and finisher (29–42d of age) phases. In each feeding trial, a total of 640 birds were randomly assigned to one of 8 treatments, with 4 replicates and 20 birds. The range in dietary Ile levels in the starter (0.23–1.14%), grower (0.21–1.05%), and finisher (0.19 to 0.98%) phases were produced using the dilution

technique, where a summit diet (high protein) was graded diluted with a nitrogen free diets to obtain the range in Ile levels above described. Data were analyzed as one-way ANOVA, and body weight gain and feed conversion ratio were regressed against Ile intake (g/kg) to estimate the levels which optimized both performance traits. Data were fitted to broken-line (BL), quadratic-broken-line (QBL) and polynomial quadratic (PQ) models. The model which better fitted data were chosen based on Akaike (AIC) information criterion. Based on such information, the estimates from PQ model was used to estimate the optimum Ile intake. Body weight gain were maximized at 1.03, 0.84, and 0.78%, while the FCR were optimized at 1.08, 0.84, and 0.73%, in the starter, grower and finisher phases, respectively. Male broiler requirement for Ile in the starter, grower, and finisher phases is 1.03, 0.84, and 0.78%, respectively.

Key Words: branched chain amino acid, factorial method

415P Effects of NSP-degrading enzymes on intestinal transit time, ileal digestibility, and cecal thermal profile of broilers fed on corn-based diets. G. Pasquali*, A. C. Neto, D. Souza, E. Muro, J. Denadai, J. Batistioli, L. Zanetti, L. Dornelas, R. F. Netto, R. G. A. C. Araujo, T. Santos, J. Sartori, and A. Pezzato, *UNESP, Botucatu, São Paulo, Brazil.*

Xylanase can breakdown arabinoxylans into arabinoxylooligosaccharides, which in turn might be fermented by bacteria present in the gut, changing the short-chain fatty acid profile in the ceca and increasing the release of peptide YY, the hormone that can slow down the intestinal transit. Thus, the effects of a xylanase and β-glucanase enzyme product supplementation on the small intestine transit time, ileal digestibility and cecal thermal profile of broilers were investigated. A total of 744 one-day-old male Cobb chicks were randomly assigned in a 2 × 2 factorial arrangement (high or low AMEn × 0 or 100 ppm of the enzyme product per kilogram of feed). Each treatment included 6 replicates with 31 broilers per floor pen. The diets were based on corn and soybean meal and formulated to contain: High AMEn, 3,025 kcal/kg (1 to 21d) and 3,125 kcal/kg (22 to 35d); or Low AMEn, 2,925 kcal/kg (1 to 21d), and 3,025 kcal/kg (22 to 35d). Chromium oxide was added to diets as an indigestible marker. At 35 d post-hatch, sampling was carried out on 4 birds per pen and 2 birds per pen for intestinal mean retention time (MRT) and cecal thermal profile, respectively. Intestinal contents from proximal jejunum, distal jejunum, proximal ileum, and distal ileum were collected to determine the mean retention time of digesta. The intestinal contents from the 4 birds per pen were pooled and the distal ileum contents were used to determine the energy and protein digestibility. Immediately posteuthanasia, the ceca was exposed and imaged using a Testo 875–1i Thermal Imaging Camera and the thermal images were processed using Testo IR Soft software. Data were subjected to 2-way ANOVA using the GLM procedure of SAS 9.3. Statistical differences were considered significant at $P < 0.05$ and the means were separated with Tukey's test. Enzyme supplementation increased the MRT of digesta in the distal ileum (125 min vs. 86 min) and improved the ileal energy digestibility (0.61 vs. 0.54). Broilers fed high AMEn diets presented increased MRT in the distal jejunum (57 min vs. 40 min) and improved energy and protein digestibility. The surface temperature of the ceca was significantly higher in broilers fed low AMEn diets (35.1°C vs. 34.4°C) than those fed high AMEn diets. In summary, the supplementation of an enzyme product composed by xylanase and β-glucanase does not affect the cecal temperature but increases the mean retention time of digesta in the distal ileum and the ileal energy digestibility of broilers fed corn-based diets, regardless of the dietary energy level.

Key Words: carbohydrase, enzyme, MRT, thermal imaging

416P Effect of protease, xylanase and amylase supplementation on performance and nutrient digestibility in broiler chickens. J. León-Landeros*[1], E. Martínez-Cruz[1], A. Pro-Martínez[3], F. Mera-Zúñiga[3], E. Sosa-Montes[1], B. Bello-Olivera[1], M. Tepox-Pérez[2], and F. González-Cerón[1], *[1]Chapingo Autonomous University, Texcoco, México, Mexico, [2]ENMEX, Mexico, Mexico, Mexico, [3]Colegio de Postgraduados, Texcoco, México, Mexico.*

Feeding represents the highest cost in poultry production. Exogenous enzymes could improve nutrient digestibility and performance of broiler chickens. The objective of the present study was to evaluate the effect of protease, xylanase and amylase [enzymatic cocktail (EC)] supplementation on performance and nutrient digestibility in broilers from 0 to 21 d of age. One hundred 20 Ross 308 one-day-old male broiler chickens were randomly placed into 12 battery pens (10 birds/pen) and allocated to 2 treatments with 6 replicates: T1 or control, diet (22% CP and 2,992 kcal/kg ME) without inclusion of the EC (0 g t^{-1}), and T2, diet (22% CP and 2,992 kcal/kg ME) including the EC (100 g t^{-1}). Titanium oxide (3 g kg^{-1}) was used as digestibility marker. In nutrition studies feed and excreta may be analyzed to evaluate the digestibility of feed and nutrients. All data were analyzed as a completely randomized design using the methodology of the general linear models. When the model was found to be significant ($P < 0.05$), treatment effects were separated using the *t*-test. Enzyme supplementation did not improve ($P > 0.05$) body weight gain (BWG) from 0 to 14 d of age. However, birds in T2 showed better ($P < 0.05$) BWG than T1 (458.9 ± 7.0 vs 438.5 ± 7.0 g) from 14 to 21 d of age. Feed conversion ratio (FCR) was not different ($P > 0.05$) between treatments during the experiment (0–21 d) but T2 (1.235) tended to a better FCR than T1 (1.255). Although apparent excreta digestibility (%) of dry matter (T1, 67.8 ± 1.0; T2, 68.6 ± 1.0), crude protein (T1, 60.9 ± 1.2; T2, 61.8 ± 1.2), metabolizable energy (T1, 70.5 ± 0.9; T2, 72.1 ± 0.9), calcium (T1, 71.0 ± 0.9; T2, 72.7 ± 0.9) and phosphorus (T1, 45.4 ± 1.7; T2, 46.5 ± 1.7) were not different ($P > 0.05$) between treatments, the results suggest an improvement with the enzyme supplementation. It is concluded that enzyme supplementation (protease, xylanase and amylase) improves BWG of broiler chickens in the starter phase.

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

417P Evaluation of two phytase levels on broiler performance when applying an elevated phytase mineral matrix. D. Dennehy*[1], H. Walters[1], Y. Dersjant-Li[2], M. Hruby[2], and J. Lee[1], *[1]Texas A&M University, College Station, TX, [2]DuPont Industrial Biosciences, Marlborough, United Kingdom.*

A randomized complete block design trial was conducted to determine the effects of a bacterial phytase on broiler performance during 3 phases (starter 1–10 d, grower 10–21 d and finisher 21–42 d). A total of 1200 d-old Cobb 500 male broilers were assigned to 4 dietary treatments each containing 10 replicate pens with 30 chicks per pen. The treatments consisted of a positive control (PC), negative control (NC), and NC with the addition of *Buttiauxella* phytase at 500 and 1,000 FTU/kg, respectively. The PC was formulated meeting nutrients requirement of broilers in each phase. Diets were based on corn, soybean meal and rice bran, in mash form. The separation between the NC and PC diets was a reduction of 0.187% aP and 0.199% Ca. At d10 and d21, 5 birds per replicate pen were removed and tibias collected for bone ash and bone weight determination. All data were subjected to an ANOVA using the GLM Procedure. Means were considered significantly different at $P \leq 0.05$ and separated using Duncan's Multiple Range Test. Reduced minerals levels in the NC diet decreased ($P < 0.05$) body weight and feed consumption and increased ($P < 0.05$) FCR throughout the trial compared with PC. Phytase inclusion at both doses improved ($P < 0.05$) all growth-related parameters compared with NC. Inclusion of phytase at both levels increased body weights to levels similar ($P > 0.05$) with PC throughout the experiment. Bone ash percentage and ash weight were negatively impacted ($P < 0.05$) in NC compared with PC broilers at 10 and 21 d of age. The addition of both levels of phytase increased ash weight and percentage to levels similar ($P > 0.05$) with PC. The addition of 500 FTU/kg of phytase improved ($P < 0.05$) FCR vs NC

during all dietary phases, and maintained FCR equal to PC during starter and grower phases. For overall 0–42d, phytase at 500 FTU/kg improved FCR ($P < 0.05$) vs PC. Elevating the level of phytase to 1,000 FTU/ kg further improved ($P < 0.05$) FCR compared with PC fed broilers during the starter (1.22b, 1.29a, 1.20bc, 1.18c for PC, NC, NC+500 and NC+1000 FTU phytase, respectively) and grower phases of production. These data indicated that feeding elevated dose of phytase is more beneficial in younger broilers, which may be related to extra-phosphoric effect as tibia ash was not affected by phytase doses. Additionally, these data imply that optimal phytase dose is related to dietary composition (e.g., phytate and Ca level in the diets) and age of the birds. In conclusion, *Buttiauxella* phytase at 500 FTU/kg could replace 0.187% aP and 0.199% Ca while maintaining performance of broilers. Furthermore, using a higher dose of 1000 FTU/kg is beneficial in starter and grower phases.

Key Words: phytase, broiler, dose, mineral matrix

418P Interactive effects of rice bran and multi-carbohydrase supplement on growth performance, gastrointestinal measurements and energy utilization in broiler chickens fed corn-soybean meal diet. J. Sanchez[1], A. Thanabalan*[1], T. Khanal[1], R. Patterson[2], B. Slominski[3], and E. Kiarie[1], *[1]University of Guelph, Guelph, ON, Canada, [2]Canadian Bio-Systems Inc., Calgary, AB, Canada, [3]University of Manitoba, Winnipeg, MB, Canada.*

High concentration of anti-nutritive phytate and fiber limits the use of rice bran (RB) in poultry diets. Effects of adding RB in corn-soybean meal diets fed to broilers without or with a multi-carbohydrase supplement (MC) was evaluated in a 2 × 2 factorial arrangement. The MC supplied xylanase, β-glucanase, invertase, protease, cellulase, amylase, mannanase with targeted activity level of 2,500, 300, 700, 10,000, 1,200, 24,000, and 20 U/kg of feed, respectively. A 2-phase feeding program (starter, d 0–24, 5% RB) and finisher (d 25–35, 11% RB) was used. Concentration of CP, crude fat, starch, and total dietary fiber in RB was 14.2, 14.0, 40.0 and 16.8% DM, respectively. All diets were iso-caloric and iso-nitrogenous and contained phytase (500 FTU/kg) and TiO_2 as digestibility marker. A total of 360-d old male Ross × Ross 708 broiler chicks were placed in cages based on BW (15 birds/cage) and allocated to diets (n = 6). Birds had free access to feed and water. Body weight gain (BWG) and FCR were calculated based on daily mortality and weekly BW and feed intake records. Excreta samples were collected 3-d consecutively before the end of each phase for apparent retention of gross energy (ARGE). Samples of birds were sacrificed at the end of starter (8), and finisher (7) phases for gut weight and ceca digesta for organic acid concentration. $P < 0.05$ was considered significant. Feed intake was not ($P > 0.05$) affected by diets and there was no interaction ($P > 0.10$) between RB and MC on BWG and FCR throughout. The main effects of MC was such that MC fed birds had improved BWG and FCR in the starter and finisher compared with non-MC birds. Specifically, the finisher phase final BW for birds fed MC was (1,881 *vs*. 1,778 g) and FCR (1.69 vs. 1.86) relative to birds fed non-MC diets ($P < 0.01$). Feeding RB reduced ($P = 0.02$) BWG in finisher phase resulting in lower d 35 BW. There was no effect of RB on FCR ($P > 0.10$) throughout. Although neither interaction between RB and MC or MC affected ($P > 0.10$) gizzard weight, RB increased ($P \leq 0.01$) gizzard weight on d 25 and 35. An interaction ($P \leq 0.01$) between RB and MC on concentration of propionic and iso-butyric acids in ceca digesta was such that MC reduced these acids in non-RB diet. The ARGE values for birds fed MC was higher ($P \leq 0.02$) in the starter and finisher phases. Specifically, the ARGE values were (75.7 vs. 73.3%; $P < 0.01$) and (77.2 *vs*. 75.7%; $P = 0.02$) for MC vs. non-MC birds in starter and finisher phases, respectively. In conclusion, RB increased gizzard weight and reduced final BW whereas MC improved growth and energy utilization. Reduction of iso-butyric acid due to MC in non-RB diet suggested reduced formation of protein fermentation metabolites in the ceca.

Key Words: broiler chicken, growth performance, multi-enzyme blend, microbial activity, nutrient utilization

419P Energy-releasing efficacy of a 6-phytase, Natuphos E, on broiler performance compared with fat reduction in corn soy diets. M. Coelho*[1] and P. Ader[2], *[1]BASF Corporation, Humble, TX, [2]BASF SE, Lampertheim, Germany.*

Recent studies demonstrated that higher doses of phytase can break down most of the phytic acid and release energy from the inositol ring. The objective of this experiment was to evaluate the metabolizable energy (ME)-releasing efficacy of a 6-phytase, Natuphos E, on broiler performance compared with fat in corn soy diets. A total of 2100 one-day old Cobb 500 male broilers were randomly allocated into 7 dietary treatments with 10 replicates and 30 birds each replicate and $P < 0.05$ was used to determine the level of significance. Dietary treatments consisted of positive control (PC) with all nutrients meeting or exceeding Cobb 500 nutrition manual; NC1 = PC- 0.17% Calcium (Ca), - 0.15% non-phytate phosphorus (nPP), −100 kcal/kg, NC2 = PC- 0.17% Ca, - 0.15% nPP, −200 kcal/kg and NC3 = PC- 0.17% Ca, - 0.15% nPP, −300 kcal/kg and phytase treatments were NC1+1000, NC2+2000 and NC3+3000 FTU/kg. Feed intake, body weight, body weight gain and feed conversion ratio (FCR) were recorded at d 14, 28, 42. Data were subjected to Duncan's Multiple Range Test. For the results, d42 body weight was 2955, 2856, 2776, 2642, 2966, 2993 and 3020 g, respectively; FCR was 1.726, 1.766, 1.801, 1.837, 1.725, 1.721 and 1.712, respectively. NC1, NC2 and NC3 decreased ($P < 0.05$) body weight and increased ($P < 0.05$) FCR compared with PC. NC1+1000, NC2+2000 and NC3+3000 FTU/kg Natuphos E, increased ($P < 0.05$) body weight and body weight gain, and decreased ($P < 0.05$) FCR compared with the NC1, NC2 and NC3, respectively. Body weight, body weight gain and FCR of NC1+1000, NC2+2000 and NC3+3000 FTU/kg treatment was equal ($P < 0.05$) to PC at 14, 28 and 42 d. In conclusion, 1000, 2000 and 3000 FTU/kg phytase, Natuphos E, compensated, performance wise, for the reduction of 100, 200 and 300 kcal/kg, respectively, and met or exceeded ($P < 0.05$) the body weight, body weight gain and FCR of the PC. Supplementation of 3000 FTU/kg Natuphos E could compensate, performance wise, the reduction of 0.18% calcium, 0.15% available phosphorus, 0.02% sodium and 300 kcal/kg metabolizable energy in the diet.

Key Words: phytase, metabolizable energy, broiler, performance, phytase energy release

420P Assessment of a target dose of a 6-phytase, Natuphos E, on ileal digestibility of sodium and broiler performance at variable sodium levels in corn/soy diets. P. Ader[2] and M. Coelho*[1], *[1]BASF Corporation, Humble, TX, [2]BASF SE, Lampertheim, Germany.*

The objective of this study was to evaluate the efficacy of a 6-novel phytase, Natuphos E, on ileal digestibility of sodium (Na) and broiler performance in standard industry corn/soy diets through d 14. A total of 1449 Male Cobb 500 broilers were used in a randomized complete block design pen trial (23 birds/pen × 7 phytase doses × 9 replicates) and $P < 0.05$ was used to determine the level of significance. Phytase dose rates were 0 (NC), 500, 1000, 1500, 2000, 2500 and 3000 FTU/kg with a 0.15% P and 0.19% Ca inorganic reduction. Apparent distal ileal

sodium digestibility (IDS) and bird performance at 14 d were measured. TiO2 was used as an indigestible marker. Data were subjected to one-way ANOVA. Additionally, linear and quadratic regression analysis were performed. Birds fed 500, 1000, 1500, 2000, 2500 and 3000 FTU/kg Natuphos E had higher ($P < 0.05$) IDS versus NC (−0.49, −0.38, −0.33, −0.32, −0.30 and −0.27 versus −0.78%), increased ($P < 0.05$) 14d body weights versus NC (422, 438, 440, 457, 459, 467g versus 366g, decreased ($P < 0.05$) 0–14d FCR (1.220, 1.209, 1.204, 1.195, 1.192 and 1.189 versus 1.254). Phytase supplementation increased IDS and body weight (quadratic, $P < 0.05$) and decreased FCR (quadratic, $P < 0.05$). Body weight gain was correlated with increased in IDS with a R^2 of 0.95. A subsequent trial investigated the effect of variable sodium levels, while maintaining electrolyte balance, on broiler performance in a standard industry corn/soy diets in the presence of 1000 FTU/kg Natuphos E, while removing 0.184% nPP and 0.220% Ca. A total of 1242 Male Cobb 500 broilers were used in a randomized complete block design pen trial (25 birds/pen × 6 sodium levels × 9 replicates) and $P < 0.05$ was used to determine the level of significance. Data were subjected to one-way ANOVA. Sodium (Na) levels in starter were 0.13, 0.14, 0.15, 0.16, 0.17 and 0.18%, grower 0.14, 0.15, 0.16, 0.17, 0.18 and 0.19%, and finisher 0.15, 0.16, 0.17, 0.18, 0.19 and 0.20%. Na levels were achieved by varying the levels of sodium chloride while maintaining electrolyte balance. Body weights (BW) at d14 with 0.15% Na (587g) was higher ($P < 0.05$) than 0.13, 0.17% and 0.18% Na (473, 476 and 465 g), BW at d28 with 0.16% Na (1448g) was higher ($P < 0.05$) than 0.14, 0.18 and 0.19% (1,362, 1,265 and 1,097g), and BW at 42d with 0.17% Na (2465g) was higher ($P < 0.05$) than 0.15, and 0.19% Na (2,345 and 2,214g). In summary, Natuphos E increased sodium digestibility, increase in IDS was correlated with bird weight gain and FCR. Best broiler performance was with 0.15%, 0.16% and 0.17% sodium in starter/grower/finisher.

Key Words: phytase, sodium ileum digestibility, broiler, sodium requirement

421P Effect of target dosing Natuphos E levels on apparent metabolizable energy in corn-soy diets fed to male broilers. F. Parks*[2], R. Jones[2], and M. Coelho[1], *[1]BASF Corporation, Humble, TX, [2]BASF Corporation, Florham Park, NJ.*

Recent studies demonstrated that higher doses of phytase can break down most of the phytic acid and release energy from the inositol ring. The objective of this experiment was to evaluate the apparent metabolizable energy (AME)-releasing of a 6-phytase, Natuphos E, on broiler metabolizable energy in corn soy diets. A total of 1840 one-day old Cobb 500 male broilers were randomly allocated into 8 dietary treatments with 10 replicates and 23 birds each replicate. Dietary treatments consisted of positive control (PC) with all nutrients meeting or exceeding Cobb 500 nutrition manual; negative control (NC) = PC- 0.17% Calcium (Ca), - 0.15% non-phytate phosphorus (nPP), −300 kcal/kg and phytase treatments were NC+1000, +1500, +2000, +2500, +3000 and +3500 FTU/kg. Partial fecal collection was done at the end of grower phase (d 28) by papering over the pens. Fecal material was freeze-dried via Lypholizer (FreeZone Freeze Dry Systems) and AME was calculated by the ratio of indigestible marker, using partial excreta collection. TiO2 was used as an indigestible marker. Data were subjected to one-way ANOVA. Additionally, linear and quadratic regression analysis were performed. $P < 0.05$ was used to determine the level of significance. For the results, the AME for PC, NC, NC+1000, +1500, +2000, +2500, +3000 and +3500 FTU/kg was 3148, 2838, 2944, 2984, 3033, 3080 and 3123 kcal/kg AME, respectively. NC AME decreased ($P < 0.05$) vs PC. NC+1000, +1500, +2000, +2500, +3000 and +3500 FTU/kg AME increased ($P < 0.05$) vs NC. Each phytase dose increased ($P < 0.05$) compared with the previous dose, except for NC +3500 FTU/kg. In conclusion, supplementation of NC = PC- 0.17% Calcium (Ca), - 0.15% non-phytate phosphorus (nPP), −300 kcal/kg with 1000, 1500, 2000, 2500 and 3000 FTU/kg Natuphos E phytase released 106, 146, 195, 242 and 285 kcal/kg AME, respectively. Supplementation of 3000 FTU/kg Natuphos E phytase released 285 kcal/kg AME.

Key Words: phytase, apparent metabolizable energy, broiler, corn/soy diet, indigestible marker

422P Xylanase in corn-soybean meal diets improves the growth performance of broiler chickens. M. Lemos de Moraes*[1], L. Lahaye[1], M. Barrios[1], C. Boudry[2], R. S. Brito[3], and D. P. Hernández[3], *[1]Jefo, Saint-Hyacinthe, QC, Canada, [2]Puratos, Brussels, Belgium, [3]Applied Animal Research Center, Chablekal, Yucatan, Mexico.*

Traditionally, xylanases are used in diets including cereals rich in non-starch polysaccharides; however, corn-based diets are largely used for poultry in many regions. A study was conducted to evaluate the effects of a bacterial xylanase on broiler chickens fed corn-soybean meal-based diets. A total of 1,440 Cobb male chicks were randomly distributed into 3 treatments with 8 replicates of 60 birds each. Broilers were reared from 1 to 42 d of age in floor pens. The treatments differed only on feed formulation: T1, standard diet (STD); T2, diet with 150 kcal/kg reduction in metabolizable energy (RED); and T3, RED diet supplemented with a *Bacillus subtilis* xylanase (RED+XYL, 100 g/t; Jefo & Puratos). The recommended xylanase uplift in this treatment diet is 80 kcal/kg. The goal of using a greater uplift (150 kcal/kg) was to generate a substantial drop on performance. All diets were formulated with phytase and its recommended uplift for P and Ca. Growth performance and the feed cost/kg of live weight were compared by ANOVA and Duncan statistical analyses in a complete randomized design. From d 14 until the end of the trial, birds fed the RED diet had the poorest ($P < 0.05$) results for average daily gain (ADG) and feed conversion ratio (FCR). For the overall rearing period, the reduction of 150 kcal/kg (RED diet) negatively affected ($P < 0.05$) both ADG and FCR by 4.6%, which was partially rescued ($P < 0.05$; 49% for ADG and 45% for FCR) by the addition of the xylanase to the RED diet. The RED+XYL diet had the lowest cost/kg of live weight (difference of USD 0.08 in comparison to the RED diet and USD 0.21 to the STD diet). The bacterial origin xylanase studied may be used to improve growth performance and to allow savings on feed cost when using a reduced energy corn-soybean meal-based diet in broiler chickens.

Key Words: broiler chicken, corn, energy, xylanase, growth performance

423P Performance of chickens fed diets from 1 to 21 days of age with different calcium/phosphorus ratios and supplemented with phytase. M. Costa, P. Rezende, L. Santos, T. Andrade, M. Cafe, and J. Stringhini*, *Universidade Federal de Goias, Goiania, Goias, Brazil.*

We aimed at the present experimente to evaluate the different calcium/phosphorus ratios and the supplementation of phytase on the performance of broilers from one to 21 d of age. The experiment was conducted in the Experimental Poultry Facilities of the EVZ/UFG, Goiânia/BraZil. 400 d-old chicks were housed in metallic cages, distributed in a completely randomized design and in the factorial arrangement 2x4, with 8 treatments, 5 replicates with 12 birds each. During the experimental period chicks received corn-soybean meal and corn gluten based diets. Experimental diets consisted of 2 doses of phytase (750 and 1500 FTU/kg) and 4 calcium/phosphorus ratios (2.3; 2; 1.7 and 1.4/1), considering

the ratio of 2/1 of 1.011% of calcium and 0.482% of P available (1 to 7 d) and 0.907% calcium and 0.432% p available Level (8 to 21 d), based on the levels recommended by Rostagno et al. (2017). For diet formulation the enzyme nutritional contribution proposed for phosphorus release. The enzyme was added to the feed in substitution of the inert. The variables evaluated at 21 d were, weight gain (WG), feed intake (FI), Feed conversion (FC) and final weight (FW). The data obtained were submitted to ANOVA, the averages compared by the test of Scott-Knott at 5% probability by the statistical program R. In the period of 1 to 21 d of age, the different Calcium/phosphorus ratios influenced the end of the birds (P. 0.05). The birds that received the dietetic ratio of 2.3/1 of Ca/P in presented smaller FW. Phytase supplementation influenced the final weight and weight gain of birds ($P < 0.05$). The broilers that received 1500FTU of phytase presented greater weight gain and final weight (P. 0.05). The different Calcium/Phosphorus ratios and the supplementation of phytase didn't influence the FI and FC (P. 0.05). The reduction of calcium supplementation and the use of 1500FTU of phytase improves the weight gain of chickens until 21 d of age.

Key Words: enzyme, mineral, phytic acid, starter diet, super dosing

424P Performance of broilers fed diets with protease. A. L. Carneiro, G. Carvalho, J. Martins, L. P. Gomides, F. Carvalho, M. Cafe, and J. Stringhini*, *Universidade Federal de Goias, Goiania, Goias, Brazil.*

The reduction of the protein level of the ration is a way to customize the costs of the ration production, because of the most costly nutrient after energy. Another way to moderate the ration costs is to increase the digestibility of the protein of the various ingredients with the use of exogenous proteases. Thus, in this experiment, we aimed to evaluate diets with nutritional adjustments, and supplementation with the enzyme protease on broiler performance. 1280 male Chicks Cobb 500 with a day of age were housed at the Aviary School of UFG with 5 treatments (T1. Ration control, T2. Ration with the nutritional profile proposed by the company, T3. Ration with twice the nutritional profile proposed by the company, T4. T2 with addition of protease, 5. T3 with addition of protease). The basal ration consisted mainly of soybean and maize bran and the addition of 0.05% protease of *Bacillus licheniformis* PWD-1 (Cybenza DP100). Broilers and diets were weighed weekly, to calculate feed intake (FI), Weight gain (WG) and average final weight (FW), both in grams and calculated the food conversion (FC) corrected by mortallity. The experimental design adopted was a randomized block design in 2 × 2 + 1 factorial arrangement, with 8 replications with 32 birds each, totaling 1280 birds. The data were analyzed by ANOVA through the R program, and compared by Tukey test (5%). no significant interaction between treatments were observed for performance variables ($P < 0.05$). Considering the period from 1 to 7 d there was a difference ($P < 0.10$), in which animals fed rations with enzyme obtained better FW final and best WG. For the FI in the pre-starter phase, the result was statistically different, in which the average of the treatments with the valorization proposed by the company and protease addition showed lower feed intake compared with control group. In the period of 1 to 21 d, regardless of the enzyme nutritional matrix valuation, with or without enzyme, the treatment control had lower FI and better FC. Already considering the whole period of the experiment from 1 to 42 d, no statistical difference was observed, because after the 21 d the birds adapted to the diet, with or without enzyme, obtaining the same results as the treatment control. It is possible to conclude that diets with reduction in the nutritional matrix considering the addition of protease is possible to use in pre-starter and starter phase, with satisfatory performance.

Key Words: exogenous enzyme, feed conversion, feed intake, nutrition, weight gain

425P Enhancing nutrient utilization in corn for broiler chickens through supplemental phytase. T. Woyengo[1], K. J. Bogota*[1], J. Wilson[2], and A. Cowieson[2], *[1]South Dakota State University, Brookings, SD, [2]DSM Nutritional Products, Kaiseraugst, Switzerland.*

The objective was to determine the effects of increasing level of phytase in corn-based diet for broilers from 1,000 FTU/kg (the industry recommended level) to 2,500 FTU/kg (super dose level) on apparent ileal digestibility (AID) of nutrients, and dietary AMEn value. One hundred and 80 male broiler chicks were divided into 30 groups and fed 3 diets in a completely randomized design (10 groups/diet) from d 15 to d 21 of age. The diets were positive control (PC) diet with or with no added phytase, and negative control (NC) diet with phytase at 1,000 or 2,500 FTU/kg. The PC diet was formulated to meet or exceed the NRC (1994) recommended nutrient requirements for broiler chickens except for ME, which were reduced by 150 kcal/kg. The NC diet was same as the PC diet except for Ca and non-phytate P contents, which were reduced 0.18% and 0.15%, respectively. Apparent ileal digestibility (AID) of DM, GE starch, CP, Ca, and P; and apparent retention DM, GE, and CP were calculated using the indicator method (Eq. [2]; Stein et al., 2007). The AMEn value for diets was calculated as described by Hill et al. (1960). Birds fed the PC diet had greater ($P < 0.05$) AID of GE and CP than birds fed NC diet. The AID of starch, Ca and P for PC diet did not differ from those for NC diet. However, the AMEn for PC diet was lower ($P < 0.05$) than that for NC diet (3,189 vs. 3,456 kcal/kg). An increase in level of phytase in the NC diet from 1,000 to 2,500 FTU/kg resulted in an increase ($P < 0.05$) in AID of GE and CP by 2.5 and 7.1%, respectively, and tended to increase ($P = 0.082$) the AID of P by 10.4%. An increase in level of phytase in the NC diet from 1,000 to 2,500 FTU/kg did not affect the AID of starch and dietary AMEn value. In conclusion, an increase in level of phytase in a low energy, Ca and non-phytate P corn-based diet improved ileal digestibility of GE, CP, and P. The apparent retention of GE and AMEn values for PC diet were lower ($P < 0.05$) than those for the NC diet, and just like for AID of GE, the reason for the greater apparent retention of GE and AMEn values for PC diet than for the NC diet is not clear. This indicates that supplementation of phytase at super dose levels (≥2,500 FTU/kg) is more beneficial with regard to improving ileal digestibility of energy, protein, and P in corn for poultry than supplementation of phytase at the industry recommended level.

Key Words: broiler, corn, nutrient digestibility, high level of phytase, enzymes

426P Effect of xylanase on apparent metabolizable energy and apparent ileal digestibility of amino acids: Meta-analysis approach. M. Zouaoui*, F. Guay, and M.-P. Létourneau-Montminy, *Université Laval, Quebec, QC, Canada.*

The key of nutrition consists in the combination of feed formula and ingredients to maximize nutrient utilization and optimizing feed cost. Feedstuffs of plant origin used in broiler production contain indigestible substances. In addition to phytic acid, carbohydrates known as non-starch polysaccharide (NSP) are considered low digested by broilers. Xylanase was known to release energy from the fibrous portion of cereals. Therefore, energy should be the most sensitive parameter for assessing the value of xylanase. The objective of this study is to evaluate the effect of xylanase supplementation on apparent metabolizable

energy (AME) and amino acid (AA) apparent ileal digestibility (AID) in broilers through meta-analysis approach. Forty articles published between 1997 and 2018 and including 196 experiments were used. Multiple regression models were fitted with the random effect of the experiment. Xylanase supplementation increases linearly AME (P = 0.01, R^2 = 0.87); an improvement of 0.90 MJ kg^{-1} DM was estimated with 2000 XU kg^{-1}. In addition to improving the digestibility of AME, xylanase linearly improved the AID of all essential amino acids (P < 0.001: Thr, R^2 = 0.61; P < 0.01: Ile, R^2 = 0.77, Leu, R^2 = 0.79, Lys, R^2 = 0.91, Met, R^2 = 0.91, Phe, R^2 = 0.85, Ser, R^2 = 0.83; P < 0.05: Arg, R^2 = 0.85) and tended to increase linearly AID of His (P = 0.07,R^2 = 0.88) and Val (P = 0.08, R^2 = 0.76). The incorporation of 2000 XU kg^{-1}generates an increase of AID from 2% for Thr to 0.7% for Val. These improvements in AA AID with xylanase may reflect reduced endogenous AA losses resulting from the reduction of the anti-nutritive effects of NSP. The current study showed that xylanase supplementation affected positively AA AID and AME but this effect may vary depending on the composition of the diet. The current models allow quantifying the effect of xylanase on AME and AA which is important for broiler nutrition and feed industry.

Key Words: broiler, amino acid, apparent metabolizable energy, xylanase, meta-analysis

427P Efficacy of phytase superdosing on growth performance, pancreatic response and nutrient digestibility of broilers with a high trypsin inhibitor diet. Q. Zhang*, F. Yan, J. Chen, K. Wedekind, and M. Vazquez-Anon, *Novus International Inc., St. Charles, MO.*

A battery study was conducted to evaluate the efficacy of phytase superdosing in improving growth performance, pancreatic response, and nutrient digestibility of broilers with a high trypsin inhibitor model. There were 5 dietary treatments: positive control (PC), negative control 1 (NC1), NC1+ 1500 U/kg phytase, negative control 2 (NC2), and NC2 + 1500 U/kg phytase. In NC1 and NC2, the SBM was substituted with full fat extruded soybeans to target the trypsin inhibitor level at 2.5 mg/g diet. The formulated Ca and non-phytate P (nPP) for PC, NC1 and NC2 were: 0.93% and 0.45% (normal), 0.93% and 0.45% (normal), and 0.78% and 0.30% (marginal deficiency), respectively. The source of phytase was CIBENZA PHYTAVERSE (Novus International Inc., St. Charles, MO). Each diet was fed to 9 replicate pens of 8 birds from 8 to 15 d in mash form. On d 14, samples from 1 bird/pen were collected for activities of trypsin and chymotrypsin in jejunal digesta. On d 15, all birds were sacrificed for pancreas weight, and ileal digesta for nutrient digestibility. Data were analyzed by one-way ANOVA and means were separated by protected Fisher's LSD test with a value of ≤0.05 considered significantly different. Compared with PC, trypsin inhibitors (NC1) significantly decreased feed intake, reduced digestibility of DM, CP and AA, inhibited trypsin activity in jejunal digesta and increased pancreas weight; phytase supplementation to NC1 significantly increased P digestibility and had no significant effect on other measurements. Compared with NC1, P deficient diet (NC2) decreased body weight gain and feed intake, increased feed to gain ratio, decreased DM digestibility and decreased chymotrypsin activity in jejunal digesta. Phytase superdosing significantly reversed all the changes induced by P deficiency, and also increased P digestibility, decreased the pancreas weight and increased digestibility of Cys (4.5%) and Met (2.2%) compared with NC2. Overall, with a high trypsin inhibitor model, phytase superdosing significantly increased P digestibility, regardless of P deficiency; and it also improved growth performance, pancreatic response and digestibility of DM, Cys and Met at marginal P deficiency.

Key Words: phytase superdosing, trypsin inhibitor, growth performance, nutrient digestibility, pancreatic response

428P Increasing the omega-3 content of hen's eggs through dietary supplementation with the microalgae *Aurantiochytrium limacinum* (All-G-Rich). C. Moran[1], M. Morlacchini[2], T. Ao*[3], J. Keegan[1], and G. Fusconi[2], *[1]Alltech SARL, Vire, France, [2]CERZOO S.r.l, Piacenza, Italy, [3]Alltech Inc., Nicholasville, KY.*

The omega-3 fatty acid (n-3 FA) content of eggs can be increased through dietary supplementation of hens with n-3 FA-rich microalgae. The objective of this study was to investigate the temporal pattern of enrichment of hen's eggs following dietary supplementation with microalgae at different inclusion levels. The study was conducted using 360 Isa Brown laying hens (130/140 d old) with 10 birds per pen. Pens were randomly allocated to one of 4 dietary groups, each having 9 replicates. The diets included one untreated control and 3 treatment diets supplemented with docosahexaenoic acid (DHA)-rich microalgae (All-G-Rich) at a rate of 0.25%, 0.5%, and 1.0%. The microalgae supplement consisted of a heterotrophically grown, unextracted *Aurantiochytrium limacinum* biomass (AURA; CCAP 4087/2; Alltech Inc., KY, USA). Fatty acid profiles of the egg yolks on each sampling date (d 0, 7, 14, 21, 28, 56, 84, 112, 140 and 168) were determined using a pooled sample of 3 egg yolks/pen. Differences between the groups in terms of productivity and DHA content were established using the repeated measured ANOVA ($P < 0.05$). For each treatment temporal egg DHA content data were fit to a quadratic plateau regression model to estimate the point after which the DHA concentration stabilized. The inclusion of AURA had no effect on any of the productivity parameters measured. Overall, each increase in inclusion level resulted in eggs with a significantly higher DHA content. No plateau point was observed for the Control group. The egg DHA content increased until d 43 (95% C.I. ± 26, 156), 35 (95% C.I. ± 27, 48) and 24 (95% C.I. ± 19, 28) for the 0.25, 0.5, and 1.0% algae treatments, respectively. Once the plateau point was reached the DHA content of the eggs remained stable for the remainder of the experimental period. A similar efficiency of DHA transfer from the diet to the egg was observed for the 0.25 and 0.5% algae groups (28 and 26%, respectively) while the 1.0% group had a lower transfer efficiency (20%, $P < 0.05$). Egg EPA content increased significantly with each increase in inclusion level. An increase in n-3 FA and a decrease in n-6:n-3 ratio was observed with each increase in algae inclusion level ($P < 0.05$). These results indicate that dietary supplementation of hens with AURA is an effective method by which to increase egg EPA and DHA concentrations in addition to reducing the n-6:n-3 ratio making the eggs more nutritionally valuable. In addition, eggs were enriched in proportion to the dietary inclusion level and reached a stable DHA concentration earlier at higher inclusion levels.

Key Words: algae, layer, DHA, EPA, omega-3

429P Administration of refined functional carbohydrates with yeast culture improves growth performance and reduces pathogen colonization in broiler chickens. L. Froebel*[1], S. Jalukar[2], T. Lavergne[2], J. Lee[1], and T. Duong[1], *[1]Texas A&M University, College Station, TX, [2]Church and Dwight, Mason City, IA.*

Prebiotics are thought to be potentially important alternatives to antibiotic growth promoters in poultry production because of their beneficial health effects. The administration of prebiotic Refined Functional Carbohydrates (RFC) derived from the cell wall of *Saccharomyces cerevisiae* has been demonstrated to improve animal health, growth performance, and microbial food safety in poultry production. In this study, we evaluated the effects of a prebiotic product composed of RFCs with yeast culture on growth performance and gastrointestinal and environmental microbiota when administered in feed and water to broiler chickens. Broilers were fed rations supplemented with a low (50 g t^{-1}) or high (100 g t^{-1}) dose of prebiotic and administered untreated or prebiotic-treated water in the final 72 h of production. Broilers administered untreated or BMD-treated feed and untreated water served as control groups. Growth performance was evaluated through 42 d of production, while gastrointestinal and litter microbiota were evaluated through the production period and 8 h after feed withdrawal. Growth performance and bacterial counts were analyzed using ANOVA, and pathogen prevalence was analyzed using the χ^2 test. Administration of either prebiotic dose increased final body weights ($P < 0.05$) by up to 297 g and improved average daily weight gain ($P < 0.05$) by up to 14 g per bird-day when compared with the antibiotic or untreated control diets. Prebiotic administration increased counts of total Lactic Acid Bacteria at d 21 ($P < 0.05$) up to 0.8 $\log_{10}$ cfu g^{-1} and decreased counts of *Campylobacter jejuni* at d 42 ($P < 0.05$) up to 0.96 $\log_{10}$ cfu g^{-1} in comparison to the control groups. Although the effect was not significant ($P > 0.05$), the prevalence of *Campylobacter* in the cecum after feed withdrawal was 10% lower when broilers were administered feed containing the high dose prebiotic as compared with the other treatments. Additionally, although not significant ($P > 0.05$), recovery of *Campylobacter* from the litter was up to 50% lower when broilers were administered prebiotic as compared with the control treatments. These data suggest that co-administration of RFCs with yeast culture as a prebiotic can be used to improve growth performance, promote populations of beneficial bacteria, and reduce human foodborne pathogens in poultry.

Key Words: prebiotic, refined functional carbohydrate, Saccharomyces cerevisiae, *Campylobacter*, lactic acid bacteria

430P The effect of Poulk (*Stachysschtschegleevii*) on performance, blood parameters and immune system of broiler chickens. A. Mozafari, S. Rahimi*, and M. Ayyari, *Tarbiat Modares University, Tehran, Iran.*

A study was conducted to evaluate the effect of Poulk (*Stachysschtschegleevii*) as a herbal extract and antibiotic on growth performance, immune system and blood factors in broiler chickens. A total of 3 hundred one day old broiler chicks (Ross 308) were randomly distributed into 5 treatment groups using the completely randomized design, each treatment consisting of 4 replications with 15 chicks per replicate.The groups were assigned to receive the treatment diets as follows: basal diet with no added the poulk extract nor the antibiotic as the control group, basal diet supplemented with 0.5%, 1% and 1.5% poulk extract in drinking water and basal diet supplemented with 150 g/ton of chlortetracycline 50%. During the experiment feed and water were provided ad libitum. Feed intake, BW and FCR were recorded weekly. At last day of experiment (d 42), 2 birds per replicate were euthanized humanely using CO2 gas. Carcass characteristics and relative organs weight were measured. The data were analyzed by using the GLM procedure of the statistical software (SAS Institute Inc., 1989, version 9.00). Mean differences among treatments were evaluated through Tukey test at $P < 0.05$. FI was highest in control group but lowest in antibiotic fed group ($P > 0.05$).Weight gain increased by supplementation of Poulk extract, but the difference was not statistically significant. The highest and the lowest body weight were related to antibiotic group and the group which received 0.5% poulk extract, respectively ($P > 0.05$). Feed conversion

rates was significantly ($P < 0.05$) influenced by dietary treatments, such that the antibiotic and control grope had the lowest and highest FCR, respectively. The mortality rate was highest in control group. Relative weight of spleen in the 1.5% poulk extract group showed a significantly increase compared with other groups, while the relative weight of bursa Fabricius was unaffected by treatments. Newcastle disease vaccine (LaSota) was administrated with drinking water at 28 d of age. Blood samples were collected on 7and 14 d after vaccination and antibody response was determined by using HI method. The results did not show significantly difference among the treatments. It was concluded that supplementation of poulk extract (1.5%) in drinking water marginally improved BW and FCR.

Key Words: Poulk, antibiotic, broiler, performance

431P Effect of *Bacillus subtilis* on performance and intestinal lesion score of broilers challenged by *Clostridium perfringens*.
J. Hu*[1], S. Bai[1], K. Zhang[1], Q. Zeng[2], J. Wang[2], H. Peng[2], and X. Ding[2], *[1]Animal Nutrition, Wenjiang, Chengdu, China, [2]Sichuan Agricultural University, Chengdu, China.*

The experiment were conducted to study the effect of *Bacillus subtilis* (Alterion) on performance and intestinal lesion score of broilers challenged by *Clostridium perfringens* (Cp).The animal study wasconducted on broiler chickens (Ross 308) for the periodof42d using a completely randomized design.Fourhundredeighty1-d-old male broilerswereallocated to 5treatmentsgroups.Each treatment group contained8replicates with 12birdsper replicate.The 5 treatment groups were an uninfected control (CON), an infected control (CP), an infected group supplemented with enramycin (10 mg/kg, EN), an infected group supplemented with *B.subtilis* (10mg/kg, containing 10^8cfu/kg, BS), and an infected group supplemented with enramycin (5mg/kg) and *B. subtilis* (10mg/kg) (EN+BS).The broilers of the 2 to 5 groups were given a total of 3×10^4oocysts of mixed strains of *Eimeria*species on d 14 followed by orally challenged with Cp (10^8cfu/ mL/bird) on d 16 through 18 of trial. All data were analyzed by ANOVA. The results showed that there were no significant difference in BW, BWG and FI of the broilers between d 1 and 21. Compared with CON, after challenged with*Cp*, FCR was increased. FCR ratio tended to be numerically higher in CP versus other treatments ($P = 0.051$). Compared with CP, FCR was decreased in EN, BS and EN+BS. In the stage of 22 to 28 d, BW, BWG and FI were higher for CON, EN, BS and EN+BS versus CP ($P > 0.05$), and FCR were lower ($P > 0.05$). During the whole period of 1 to 42 d, FCR was significantly lower in the EN group and EN+BS group compared with the CON,CP and BS group ($P = 0.026$), there were no significant difference in BWG and FI among treatments. On 21 d, the scores of jejunal lesion in CP was the highest, and the CON group was the lowest. The score of EN, BS and EN+BS were lower than CP. On 28 d, the score of jejunal lesion in CP was still the highest, and the score of the CON, BS and EN+BS groups was lower than CP group.It is concluded that the supplementation of *Bacillus subtilis* improved performance and intestinal lesion in broiler birds.

Key Words: *Bacillus subtilis*, broiler, performance, intestinal lesion score, *Clostridium perfringens*

432P Effect of use of annatto (*Bixa orellana*) on the internal quality of eggs.
D. Malheiros*, V. Moraes, P. Ferket, K. Anderson, and R. Malheiros, *NC State University, Raleigh, NC.*

Annatto tree (*Bixa orellana*) is a large, quick-growing shrub, native to tropical America. A very dense pigment coating the seed can be extracted; the extract is called annatto. Once used as a dye for natural fibers, annatto is now used almost exclusively for coloring foodstuffs and wax polishes. At high concentration this pigment is orange, but at very low concentration it appears yellow. The objective of this study was to test the effect of dietary inclusion of this pigment for laying hens on external and internal egg characteristics. Forty-eight Hy-Line Brown 60-week-old hens were housed individually and divided into 4 dietary treatments: Control (C.), Pixafil LZ (PLZ), Annatto 0.6% (An0.6), and Annatto 1.2% (An1.2). Feed intake and egg production were recorded every day during an 8 week period, and egg quality evaluation was performed on sampled eggs every week. To analyze the results, JMP 13.0 was used to the statistical analyzes, and the means compared by Tukey test with a probability of 0.05%. Egg per hen and FCR (gEegg/gFeed) were not affected ($P > 0.05$). Egg weight and shell strength were also not affected ($P > 0.05$). Haugh Unit tended to improve by the highest dietary level of Annatto (An1.2) ($P = 0.0640$), as was albumen height ($P = 0.0129$) and shell elasticity ($P = 0.0064$). Yolk color was more yellow for PLZ, followed by An1.6, An0.6, and Control ($P < 0.0001$). Shell thickness was greatest for the eggs from C, followed by An0.6, P, and An1.2 respectivelly 0.352, 0.350, 0.340, and 0.336 mm ($P = 0.0168$). In conclusion, the use of Annatto seed extract as an alternative for yolk pigmentation shows improvement in yolk color, and also can improve the internal egg quality, specially correlated with egg albumem quality.

Key Words: annatto, egg quality, yolk pigmentation

433P Evaluation of increasing levels of spray-dried animal plasma in broiler diets.
H. Walters*[1], M. Williams[1], C. Johnson[1], J. Campbell[2], and J. Lee[1], *[1]Texas A&M University, College Station, TX, [2]APC Inc., Ankeny, IA.*

The objective of the current study was to evaluate the impact of increasing levels of spray-dried animal plasma (SDAP) during the starter phase on broiler performance. The experimental design consisted of 5 dietary treatments with 8 replicates per treatment and 33 birds per replicate for a total of 1,320 male broilers. All diets were corn-soybean meal based with treatments including a control being void of animal plasma, and SDAP administered in the starter phase only at 0.5, 1.0, 1.5 and 2%. Prior to placement, broilers received a coccidiosis vaccine via a commercial spray cabinet and were placed on built up litter for a 42-d assay period. Average body weight (BW), mortality adjusted feed conversion ratio (FCR), feed consumption, and mortality (%) were determined on d 10, 28, and 42. On d 10 and 42, birds were individually weighed to determine flock uniformity by calculating replicate coefficient of variation. Data were analyzed via a one way ANOVA with means being deemed significantly different at $P \leq 0.05$. Regression analysis was conducted to determine slope impact of increasing levels of SDAP. Increasing SDAP inclusion during the starter period elevated BW when compared with the control diet with the addition of SDAP at 2% yielding the highest BW on d 10. This improvement in BW continued through d 28 and 42 with the supplementation of SDAP at 1 and 2% yielding a 63 and 57 g improvement respectively, compared with the control on d 42. Weight adjusted FCR was influenced with the supplementation of SDAP at 1 and 2% reducing ($P < 0.05$) FCR compared with diets without SDAP. Although feed consumption was only impacted during the grower phase, birds fed SDAP at 1 and 2% had an increase ($P < 0.05$) in feed intake of 3.4 and 3.8 g per bird per day respectively, compared with birds fed the control. Regression analysis indicated linear increases in BW with increasing levels of SDAP at all evaluated time points. These results indicate that inclusion of SDAP during the starter period positively impacts broiler growth performance and that the benefits carry through to market age.

Key Words: broiler, animal plasma, performance

434P Effect of dietary silver acetate and antibiotic on broiler performance, apparent ileal digestible energy and total bone mineral content. A. Alsadwi*, H. Leyva-Jimenez, K. Gardner, Y. Al-Jumaa, R. Abdaljaleel, M. Al-Ajeeli, and C. Bailey, *Texas A &M University, College Station, TX.*

This study was conducted to evaluate the potential effect of silver acetate (AgAc) supplemented feed on broiler performance, energy ileal digestibility, and total bone mineral content compared with an antibiotic growth promotor. A total of 680 male day-old broiler chicks were allocated to 5 dietary treatments (8 replicates with 17 birds each) using a complete randomized block design. This study utilized a 3-phase rearing program (21-d starter, 14-d grower, and 7-d finisher). The dietary treatments were: unsupplemented diet (PC, with no antibiotic), bacitracin methylene disalicylate-supplemented diet (BMD, 50 ppm), and 3 dietary concentrations of AgAc: 10, 50, and 250 ppm. Body weight and feed consumption were collected at the end of each phase and broiler productivity index was calculated. At d 42, ileal digesta was collected from 2 birds/replicate for apparent ileal digestible energy, and 8 birds/treatment were obtained for Dual x-ray Absorptiometry (DXA) scan analysis to measure total bone mineral content (BMC). Data were analyzed as a one-way ANOVA, and significant means were separated using Duncan's Multiple Range Tests ($P \leq 0.05$). Dietary supplementation of AgAc at 10 and 50 ppm did not show different effects on BW and WG compared with the PC diet and BMD treatment group during the starter phase. In the grower phase, AgAc at 50 ppm reduced ($P < 0.05$) BW and productivity index compared with the BMD treatment group. Dietary AgAc at 250 ppm reduced BW ($P < 0.05$) compared with the PC diet, BMD, and dietary AgAc at 10 and 50 ppm in all phases of production. Dietary AgAc at 250 ppm also reduced ($P < 0.05$) the productivity index in starter, grower, and finisher phases when compared with the PC diet, BMD and AgAc at 10 ppm. There were no differences among treatment groups in apparent ileal digestible energy. At d 42, DXA scan analysis showed that the highest level of AgAc (250 ppm) reduced BMC ($P < 0.05$) compared with AgAc fed at 10 ppm. Overall, using dietary silver as silver acetate in broiler chicken diets could exhibit adverse effects at concentration 250 ppm, and low concentrations did not show improvement in productive parameters over the PC or similar to BMD, these could be limitations for using this form of silver as an antibiotic alternative.

Key Words: silver ion, broiler, antibiotic, mineral, alternative

435P Enrichment and concomitant storage stability of docosahexaenoic acid (DHA) enriched broiler meat: A pilot study. J. Apajalahti[1], K. Vienola[1], C. Moran[2], J. Keegan[2], and T. Ao*[3], [1]*Alimetrics Ltd., Espoo, Finland,* [2]*Alltech SARL, Vire, France,* [3]*Alltech Inc., Nicholasville, KY.*

The aim of this study was to evaluate the effect of 3 different dietary inclusion levels of a docosahexaenoic acid (DHA)-rich microalgae (AURA) on broiler performance and the enrichment of tissues with omega-3 fatty acids (n-3 FA). A secondary objective was the investigation of DHA stability in enriched tissue samples when stored at −20°C over 6 mo. The AURA microalgae supplement consisted of an *Aurantiochytrium limacinum* biomass (CCAP 4087/2; All-G-Rich, Alltech Inc., KY) containing 70% crude fat and 17% DHA. The study was conducted using 352 birds, with 11 birds per pen. Pens were randomly assigned to one of 4 treatments, with each treatment replicated 8 times. The treatments included one unsupplemented control (T1) and 3 wheat-soya based experimental diets supplemented with AURA at a level of 0.5% (T2), 1.5% (T3) and 2.5% (T4) for the starter, grower and finisher periods. Birds were weighed on d 0, 10, 24, 35 and 41, with feed intake recorded per pen. On d 41, 2 birds per pen were euthanised and individually weighed. Thigh, breast, liver and skin samples were taken post-mortem, freeze-dried and DHA content quantified following fat extraction and methylation by GC-FID (AOAC 996.06 method) at 0, 3 and 6 mo. Performance and tissue data were analyzed by ANOVA with Dunnett's (2-sided) post-hoc test to determine the differences between the mean values for each treatment. Dietary supplementation with AURA had no effect on body weight or feed intake during any period of the study. For each tissue/organ, DHA increased linearly ($P < 0.05$) with increasing level of dietary AURA (Breast: 43, 244, 290, 424; thigh: 16, 202, 469, 836; skin: 15, 205, 560, 1209; liver: 62, 514, 835, 1100; kidney: 43, 244, 290, 424 mg DHA /100 g tissue/organ for 0%, 0.5%, 1.5% and 2.5% treatments respectively). The concentration of DHA in freeze-dried, refrigerated samples was found to decrease after 3 and 6 mo, with no differences in the rate of decrease observed between algae inclusion levels or tissue/organ type. Higher inclusion levels corresponded to higher DHA concentrations after 3 mo storage for breast and thigh samples. Results for stored skin and liver samples were more variable, with no differences observed between each treatment group after 3 and 6 mo storage. The study demonstrated the potential of enriching broiler meat and organs with DHA by feeding AURA. However, the decreased stability of DHA in the tissue samples on freezing at −20°C highlights the need to extract and analyze samples as close to sampling time point as possible.

Key Words: algae, broiler, performance, DHA enrichment, DHA stability

436P Investigation of trehalose supplementation on reduction of *Salmonella* Typhimurium from broiler farming. Y.-H. Wu*[1], Y.-D. Wu[1], J.-W. Chen[2], C.-H. Chou[1], and Y.-C. Chen[1], [1]*National Taiwan University, Taipei City, Taiwan,* [2]*Council of Agriculture, Executive Yuan, Taipei, Taiwan.*

Trehalose (Treh), as a prebiotic in broiler farming, may reduce S. Typhimurium (S.T.), and it provides a potential strategy to respond the trend of global antimicrobial growth promoter prohibition. In fact, Salmonellosis in broiler not only results in the higher economic cost in poultry industry but also enhance the risk of food cross-contamination. Treh had widely used in cosmetic, food, pharmaceutical industry, but the specific activity of Trehalase in broiler was not detectable after a 21-d-old birth. Moreover, it had reported that Treh significantly increases the growth of bacteriocin-producing lactic acid bacteria rather than other in-use prebiotics. Treh was added up to 10% in diets of broilers, and it was not defective. Thus, Treh supplementation may be useful in reducing S.T. in broiler farming. This study was divided into 2 experiments: the tolerance and the antibacterial test. The AA^+ broilers were used, and 1-d aged birds were purchased. In both trials, 75 broilers (5 broilers/floor pen) were used respectively. The raising parameters and diet formula were conducted according to a commercial guideline, and the water was given *ad libitum*. Broilers were housed in an isolated floor pen with rice chaff beddings and particulate air-filtered supplies. Furthermore, the proper procedure was obeyed to avoid cross-contamination. The one-way ANOVA was conducted via SAS program with significance level as 0.05. Compared with S.T. inoculated groups, a tendency was showed toward lower feed conversion ratio (FCR) in S.T. immunized broilers cotreated with 1 and 3% Treh. However, the FCR of S.T. inoculated broilers with 5% Treh supplementation significantly improved ($P < 0.05$) and even to that of Control. The S.T. amount was decreased ($P < 0.05$)

with Treh supplementation in the cecum, whereas the total lactobacillus amount was increased ($P < 0.0.5$) with 10 to hundred folds than those of S.T. inoculated group without Treh. Moreover, the levels of acetic acid were increased ($P < 0.05$) in Treh and S.T. cotreated broilers. The results indicated that the levels of ALT, A/G, TG, and uric acid were influenced ($P < 0.05$) by inoculation, and the reverse ($P < 0.05$) effects were observed in 3 and 5% Treh cotreated groups. However, the observations of cecal H&E stainings directly indicated that the 5% Treh supplementation alleviated the inflammation condition and maintained the integrity of intestinal epithelial cells against inoculation via the inhibition of TLR4-NFκB pathway. Taken together, Treh could be applied in commercial broiler farming potentially.

Key Words: trehalose, prebiotic, broiler farming, *Salmonella* Typhimurium, antibacterial test

437P High levels of quercetin aggravates the oxidative damage caused by oxidized soybean oil in broiler. Y. Dong*, J. Lei, and B. Zhang, *State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, China.*

The study was carried out to investigate whether the quercetin can ameliorate the oxidative damage caused by oxidized soybean oil in broiler. 360, 1-d-old male Arbor Acres broiler chicks were allocated into 6 groups fed 0, 100, 200, 400, 1000 ppm quercetin with oxidized soybean oil and control diet. Six chicks from each group were sacrificed on d 21 and d 40, while D-xylose absorption was carried out meanwhile. Group comparisons were determined using one-way ANOVA. P values below 0.05 were considered as significant. Serum and liver were tested for MDA and T-AOC. Based on the result of D-xylose absorption test, oxidized soybean oil improved the intestinal absorption capacity only on d 21 ($P < 0.05$). The MDA or T-AOC level of serum and liver was not significantly affected by oxidized soybean oil on day 21. However 100 ppm, 400ppm quercetin tended to attenuate the decreased of T-AOC in liver on day 40 caused by oxidized soybean oil (P=0.051). Oxidized oil didn't lead to any change of MDA of liver and T-AOC of serum on day 40. But in the group with 1000ppm quercetin and oxidized soybean oil, the MDA level of liver was increased (P<0.05) and T-AOC of serum was significantly down regulated (P<0.05). Also feed meat ratio (day 1-40) tended to be increased in the 1000ppm quercetin and oxidized soybean oil group (P=0.052) in comparison to the control group. In conclusion, slightly oxidized soybean oil may be helpful to enhance the intestinal absorption ability especially in the early stage. But high concentration quercetin aggravates the oxidative damage caused by oxidized soybean oil in broiler.

Key Words: quecetin, oxidative damage, broiler

438P Feeding a bioactive extract from *Olea europaea* to broiler chickens exerts a positive immunoregulatory effect without major effects on gut microbiota. J. Herrero-Encinas*[1], M. Blanch[2], J. Pastor[2], A. Mereu[2], I. Ipharraguerre[2], and D. Menoyo[1], *[1]Universidad Politécnica de Madrid, Madrid, Madrid, Spain, [2]Lucta S.A., Bellaterra, Barcelona, Spain.*

The present study examined the effects of bioactive compounds from a standardized *Olea europaea* extract (OE) on gut microbiota composition and immune response in broiler chickens. A total of 3 hundred and 6 1-d-old broiler chickens (Ross 308) were randomly allocated in floor pens (6 pens / treatment, with 17 birds/pen). From 1 to 21 d of age animals were fed with a common non-medicated starter diet based on wheat and soybean meal, and from 22 to 42 d of age with their 3 respective experimental diets: a basal diet with no additives (C) and the same diet with either 100 ppm of monensin (M) or 1000 ppm of OE (Lucta S.A., Spain). At the end of trial, 2 birds per pen (n = 12) were randomly selected to collect blood for measuring circulating concentration of cytokines and glycoprotein and cecal contents for assessing microbiome profile, which was done via DNA extraction and PCR amplification on the V1-V2 regions of 16S rRNA gen. Additionally, ileal mucosa was sampled to analyze gene expression of ASBT, ChREBP, SREBP1, FABP2, FABP6, Claudin1, TGF-β4, Bu-1, TLR4 and 2β, CD3γδ, IL-2, IL-6, IL8, and IFN-g markers. Analysis of variance was performed with animal as the experimental unit and diet as fixed effect. A mixed-model was used to determine differences between M and OE in gene expression relative to C and to analyze plasma biomarkers. Mean treatment effects were separated using Tukey test at $\alpha = 0.05$. Microbial raw sequencing reads were demultiplexed, quality-filtered and analyzed using QIIME 1.9.1. Feeding OE did not affect gut microbiota abundance and diversity, being the most abundant phylum *Clostridiciaceae* in C (41.7%) and M (51.1%) treatments, and *Lactobacillaceae* in OE (65.5%) treatment. Plasma concentration and the expression of IL-8 in ileum were significantly higher ($P \leq 0.05$) in birds fed the C diet compared with OE and M fed birds. Also, the expression of anti-inflammatory TGF-β4 ($P < 0.01$) and the B-cell marker (Bu-1) ($P < 0.001$) were upregulated in birds fed the OE and M diets compared with the control. No significant effects were observed for the other variables included in this study. In conclusion, the inclusion of a bioactive extract from *Olea europaea* in broiler diets appears to exert a positive immunoregulatory effect without affecting gut microbiota.

Key Words: broiler chicken, *Olea europaea*, bioactive extract, gut function, immunoregulatory

439P Feeding laying hens *Aurantiochytrium limacinum* (All-G-Rich) enriches their tissues, organs, and eggs with docosahexaenoic acid. C. Moran[1], M. Morlacchini[2], T. Ao*[3], J. Keegan[1], and G. Fusconi[2], *[1]Alltech SARL, Vire, France, [2]CERZOO S.r.l, Piacenza, Italy, [3]Alltech Inc., Nicholasville, KY.*

An increased omega-3 fatty acid (n-3 FA) consumption is beneficial to human health. With the view to increasing consumption, the objective of this study was to evaluate the level of n-3 FA enrichment of hen eggs and tissues following supplementation with docosahexaenoic acid (DHA)-rich algae. The study was conducted using 36 ISA Brown laying hens (300/320 d old) with 3 birds per pen. Pens were randomly allocated to one of 4 diets each having 3 replicates (9 hens per treatment). The diets included one untreated control (T1) and 3 treatment diets supplemented with DHA-rich microalgae (All-G-Rich) at a rate of 0.5% (T2), 1.0% (T3) and 2.5% (T4). The microalgae supplement consisted of a heterotrophically grown, unextracted *Aurantiochytrium limacinum* biomass (AURA; CCAP 4087/2; Alltech Inc., KY, USA). Hens were individually weighed on d 0 and 28. Average daily feed intake (ADFI), egg output, feed conversion ratio (FCR), and feed conversion efficiency (FCE) were calculated per pen. The DHA content of eggs was established on d 0, 21 and 28. Whole eggs were sampled for carotenoids (lutein, zeaxanthin and β carotene), fat, protein and energy content on days −1, 20 and 27. Six animals per treatment were randomly selected and slaughtered on d 29. The entire liver and both thighs, breasts, kidneys and ceca were sampled. The right side of each organ was analyzed for DHA content. Egg weight and laying were analyzed using the repeated measures ANOVA, while the remaining parameters

were measured using the one-way ANOVA. When significant differences were detected, Tukey tests were used to separate means. Treatment had no effect on performance, egg weight or laying %. Treatment had no effect on carotenoid, fat, protein or energy content of the eggs after 28 d of supplementation with AURA. By d 28, DHA was detected at a level of 60.3, 163.8, 258.9, and 410.1 mg/100g whole egg for control, 0.5, 1.0, and 2.5% algae, respectively. Each increase in the inclusion of AURA increased egg DHA ($P < 0.05$). Liver was enriched with DHA to the highest degree, with each increase in inclusion level corresponding to a significant increase in liver DHA content. The kidneys were the second most enriched, with 1.5 and 2.5% algae treatments having significantly more DHA than control and 0.5% algae treatments. Breast and thigh tissue were enriched to a similar degree by 0.5% (15.47 and 14.27 mg DHA/100g, respectively) and 1% algae (19.62 and 18.87 mg DHA/100g, respectively) with a greater difference observed between the 2 at 2.5% algae (41.72 and 32.87 mg DHA/100g, respectively). These results indicate that supplementing feed with AURA is an effective way to increase the tissue and egg DHA content of layer hens.

Key Words: algae, layer, DHA, omega-3

440P Effect of a *Bacillus* (3 strains) spore-based probiotic in improving production performance in broilers under necrotic enteritis challenge. V. Kuttappan*[1], G. Mathis[2], F. Yan[1], J. Chen[1], and M. Vazquez-Anon[1], *[1]Novus International, Inc., St Charles, MO, [2]Southern Poultry Research Inc., Athens, GA.*

Necrotic enteritis is a major cause of economic loss in the poultry industry. Although necrotic enteritis can be effectively prevented by antibiotics, the industry is moving toward an antibiotic free program. This results in the need for effective antibiotic alternatives against necrotic enteritis. The main objective of the present study was to evaluate the effect of a Bacillus (3 strains) spore-based probiotic in improving performance in broiler birds under necrotic enteritis challenge. The study included a control or no additive group, probiotic (SPORULIN at 250g/MT of feed), and BMD50 (Bacitracin Methylene Disalicylate at 500g/MT of feed). All birds were raised on battery cages (8 replicates/treatment; 8 birds/replicate) and challenged with *Eimeria maxima* (~5,000 oocysts) at 14d followed by *Clostridium perfringens* (~10^8 cfu/ml) at 19, 20, and 21d of age. Body weight gain, mortality, and feed intake were measured during 14–21d and 14–28d, while necrotic enteritis lesions were recorded at 21d. Data were analyzed using ANOVA and means were separated with Fisher's protected LSD test. There was no difference ($P > 0.05$) in body weight gain and cumulative feed intake among the treatment groups at different time points. However, BMD50 showed improvement ($P < 0.05$) in cumulative feed conversion ratio (cFCR) during both 14–21d and 14–28d, while the probiotic group showed improvement ($P < 0.05$) in cFCR only during 14–28d which was not different ($P > 0.05$) from BMD50. Furthermore, both the probiotic and BMD50 showed similar ($P > 0.05$) mean lesion scores and reduced ($P < 0.05$) severity of NE lesion scores compared with control. Probiotic reduced mortality ($P < 0.05$) when compared with control, and BMD50 reduced mortality ($P < 0.05$) compared with both control and probiotic groups. At the end of the trial, the cumulative performance index of probiotic group was higher ($P < 0.05$) than control and lower ($P < 0.05$) than BMD60. In conclusion, results from the study suggest that the *Bacillus* (3 strain) spore-based probiotic could be effective in reducing the severity of necrotic enteritis as well as improving performance in broiler birds and can be used as alternative to antibiotics.

Key Words: *Eimeria maxima*, *Clostridium perfringens*, probiotic, antibiotic, BMD60

441P Effects of antibiotic and phytogenic feed additives on performance and gut health in broiler chickens infected with *Eimeria spp*. A. Möddel*, M. Wilhelm, and T. Wilke, *Dr. Eckel Animal Nutrition GmbH & Co. KG, Niederzissen, Germany.*

The aim of this study was to compare the effects of a phytogenic feed additive with an antibiotic growth promoter program in broilers during oral infection with *Eimeria* spp. For this, 1,020 male birds (1 d old) were randomly assigned in 3 different additive groups (1. no additive, 2. antibiotic growth promoter program, 3. phytogenic additive). The phytogenic feed additive is based on hops in combination with different herbs and spices (300 g/t AntaPhyt MO). All groups had 2 different nutritional phases (starter: 1–21 d and 22–40 d), based on maize and soybeans. The groups had 10 replications (n = 34 birds/treatment) with a period of 40 d. The results were analyzed by one-way ANOVA. At d 14, all birds were orally challenged with a vaccinal strain of *Eimeria* spp. The associated infections and intestinal immune response were assessed by performance and gut health factors. The effects on intestinal health of 10 broilers per treatment were measured at 21 d. The phytogenic group showed a significantly higher daily weight gain than the antibiotic group (70.27 g vs. 68.23 g, $P < 0.023$) over the 40-d trial. Additionally, a significant lower feed intake was observed compared with the control group (4,114.4. g vs. 4,180.97 g, $P < 0.0297$). This shows that the phytogenic additive tends to improve broiler performance. All groups good showed a mortality of <5%, and liver weights showed no significant differences. Among the intestinal health parameters, birds with phytogenic additives had numerically larger intestines in comparison to the control and the AGP program (170.8, 165.8, 167.4 cm). These findings demonstrate that the tested phytogenic feed additive could be used to improve chicken performance and health of broilers challenged with *Eimeria* spp. The inclusion of AntaPhyt MO shows better effects than antibiotic treatment in terms of weight gain and the length of the intestine. Consequently, it seems to be a suitable and beneficial alternative to the antibiotic growth promoter program investigated in the study.

Key Words: broiler, coccidiosis, phytogenic additives, hops, antibiotic growth promoters

442P Efficacy of protected benzoic acid in broilers subject to *Eimeria* challenge as affected by diet type. F. Yan*, J. Chen, V. Kuttappan, and M. Vazquez-Anon, *Novus International Inc., St Charles, MO.*

It is recognized that the benefits of eubiotic feed additives are not consistently demonstrated in research trials, especially in a clean environment without any challenge. Understanding factors affecting their efficacy will facilitate mechanism of action investigation and guide practical application. A battery trial was conducted with 384 d-old male broilers to evaluate the effect of protected benzoic acid on growth performance and gut health of broilers subject to *Eimeria* challenge as affected by diet type. The study consisted of 6 dietary treatments in a 3 × 2 factorial arrangement with 3 types of diet (rye 10%, canola meal 7.5% and poultry meal 3%, and their combination) and 2 levels of protected benzoic acid (0 and 500 g/ton AVIMATRIX Novus International, Inc.). Each diet was fed to 8 replicate pens of 8 birds. All birds were orally gavaged with a coccidiosis vaccine at 10X recommended dose on d 14. Body weight, feed intake, FCR, and mortality were determined on d 7, 14, 19, and 26. On d 27, blood samples were collected for serum coloration, IL-10 and IL-4 determination. Data were subject to 2-way ANOVA to evaluate main effects and interaction; means were separated by Fisher's protected LSD test. Body weight was reduced with inclusion of 10% rye on d 7, 14, and 19 regardless of canola and poultry meal (CPM) inclusion (*P*

< 0.05). Benzoic acid increased body weight on d 7 and 14 regardless of diet type ($P < 0.05$). On d 26, without benzoic acid, birds fed CPM weighed higher than those fed the combination, and the rye fed birds weighed in between not significantly different from either; benzoic acid increased 26-d BW of broilers fed the combination by 12%, not in the other 2 types, accounting for a trend of interaction ($P = 0.09$). Up to d 14, FCR was not significantly affected by diet type, but improved by benzoic acid by 17 and 7 points on d 7 and 14 respectively ($P \leq 0.05$). There was an interaction between diet type and benzoic acid on d 19 ($P = 0.07$) and 26 ($P < 0.05$) where combining rye and CPM led to higher FCR, which was reversed by benzoic acid supplementation. Feed intake was affected by diet type on d 14, 19, and 26 ($P < 0.05$) in which higher BW typically corresponded to higher feed intake, but not significantly affected by benzoic acid ($P > 0.10$). Serum IL-4 was the highest in birds fed rye, followed by CPM and rye, and then by CPM ($P < 0.05$), indicating rye was capable of inducing inflammatory responses. In summary, protected benzoic acid improved growth performance of broilers subject to *Eimeria* challenge in all 3 diet types, and the efficacy was greater when diet complexity was increased by combining viscous grain and low digestible protein ingredients.

Key Words: benzoic acid, *Eimeria*, broiler

443P Assessment of a blended essential oil feed additive (Biolex) on broiler growth performance, nutrient digestibility, and intestinal morphology. S. Osho*[1], N. Horn[2], and O. Adeola[1], *[1]Purdue University, West lafayette, IN, [2]BioMatrix International, Princeton, MN.*

Essential oils, prebiotics, and acidifiers have been shown to improve poultry performance by impacting the gastrointestinal microbiome, immune dynamics, and gastrointestinal secretions. The aim of this study was to evaluate the effect of a blended essential oil agglomeration (Biolex; BioMatrix International, Princeton, MN) comprising a blend of prebiotic-agglomerated essential oils, lactic acid, β-glucan, and mannan-oligosaccharides on broiler growth performance, ileal nutrient and energy digestibility, and intestinal morphology. A total of 480 male broiler (Cobb 500) chicks (one-d-old, 49 ± 0.72 g) were randomly allocated to 2 treatments with 10 replicate cages per treatment (24 chicks/cage) in a randomized complete block design. The dietary treatments include a control diet [corn-soybean basal diet], and control diet supplemented with 0.1% Biolex. The experimental diets were administered from 1 to 22 d post hatching. Growth performance was recorded on weekly basis, (8, 15, and 22 d post hatching) and ileal digesta were collected for analysis of dry matter (DM), nitrogen, and Energy (E) digestibility on 8, 15 and 22 d post hatching. Bird with the median weight in each cage was euthanized and the mid ileum was excised for intestinal morphology on 8, 15 and 22 d post hatching. Compared with the control group, birds fed diets supplemented with 0.1% Biolex had a greater ($P < 0.05$) body weight (at 15 and 22 d), weight gain (15 and 22 d), and feed intake at 15 d post hatching. The improvements in weight gain are partly attributable to increased feed intake as there were no statistical improvements in feed efficiency. Birds fed Biolex had greater ($P < 0.05$) crude fat (15 and 22 d), and ileal digestibility compared with birds fed control diet. There was no effect of treatment on other ileal digestibility parameters measured and intestinal morphology. In conclusion, the results from the current study show that dietary supplementation of Biolex improves weight gain of broiler chickens, which can be partially attributed to improved ileal crude fat digestibility.

Key Words: broiler, Biolex, prebiotic, performance, digestibility

444P Evaluation of multiple levels of phosphatidic acid on growth performance and breast meat yield in broilers. E. Sobotik*[1], J. Lee[1], S. Hagerman[2], and G. Archer[1], *[1]Texas A&M University, College Station, TX, [2]ChemiNutra, Austin, TX.*

Improving feed conversion while increasing growth is a goal of any broiler nutrition program. The use of feed additives to obtain this goal has increased in recent years. Therefore, it is important to evaluate potential feed additives for not only increased performance but for the pitfalls that may accompany it. A study was conducted to evaluate the effects of feeding phosphatidic acid (PA) in the feed at differing levels on the growth and meat yield of broilers. Dietary treatments included: T1, control (CON), T2, 5 mg/bird/day of PA (LowPA), T3, 10 mg/bird/day of PA (MidPA), and T4, 15 mg/bird/day of PA (HighPA). All birds were weighed on d 14, 28, 42 and 49 to obtain average pen weights and feed conversion ratios. On d 49, 8 birds per pen were processed and carcass and breast yield were determined. Breast filets were evaluated for the presence and severity of woody breast and white striping. Data was analyzed using GLM: a significant difference was considered $P < 0.05$. Differences ($P < 0.05$) in live bird weights between the control birds (1.659kg) and all PA treatments (pooled mean: 1.731kg) began at 28 d; however, only the LowPA carried that effect ($P = 0.05$) through the conclusion of the trial (3.553 vs 3.818 kg). Overall, LowPA (1.649) and MidPA (1.694) had lower ($P < 0.05$) FCR than the CON treatment (1.741). Increased growth observed in live bird weights in the LowPA translated to increased ($P < 0.05$) overall carcass weights (2.783 vs 2.991 kg) and specifically breast filet weights (0.693 vs 0.769 kg). Yields did not differ ($P > 0.05$) but with the increased weight feeding LowPA resulted in more total breast meat. None of the doses of PA affected ($P > 0.05$) woody breast (pooled mean: 1.23) or white striping scores (pooled mean: 1.06). In conclusion utilizing dietary PA increased live bird weights, improved FCR, and increased breast filet weight. This improvement in BW and breast weight was obtained without increasing woody breast, or white striping. These data indicate that dietary PA may increase production efficiency in broilers.

Key Words: broiler, phospatidic acid, growth, breast yield

445P Ochratoxin liver deposition in broiler chicks following the administration of heterotrophically grown *Chlorella* microalgal biomass in feed. A. Yiannikouris[2], C. Moran[1], J. Keegan[1], T. Ao*[2], K. Vienola[3], and J. Apajalahti[3], *[1]Alltech SARL, Vire, Vire, France, [2]Center for Animal Nutrigenomics and Applied Animal Nutrition, Nicholasville, KY, [3]Alimetrics Ltd., Espoo, Finland.*

The accumulation of the carcinogenic ochratoxin A (OTA) mycotoxin in the liver of broiler chickens was investigated following the administration of 90 µg/kg of OTA in feed. In addition, the administration of OTA in feed amended with *Chlorella vulgaris* biomass (CHL) was evaluated to test if microalgal biomass would decrease the OTA recovery in liver by decreasing OTA uptake in the digestive tract owing to the adsorptive nature of its insoluble carbohydrates. Experimental diets included a commercial poultry basal diet composed of wheat-soy-based mash (D1); the basal diet amended with OTA (D2); the basal diet amended with a combination of OTA and either 0.5 kg/T (D3), 1.0 kg/T (D4) or 2.0 kg/T (D5) of CHL. Male Ross 508 hatchlings were fed test diets over a 21-d period. Chicks were randomly assigned to one of 5 treatment groups divided into 40 pens, with a total of 8 replicate pens per treatment with 6 birds per pen. Birds were administered D1 during a preconditioning phase of 7 d and D2-D5 after 8 d and until the end of the study. Chicks were individually weighed on d 1 and 21. At the end of the study, all birds were sacrificed and their livers were collected and weighed. Two

pools of 2 livers were homogenized out of 4 livers collected per pen for the quantitative analysis of OTA content. A total of 5g of liver homogenate was extracted, and OTA was chromatographically separated and quantified using RPC18-HPLC equipped with a fluorescent detector. Results showed that zootechnical performances and birds characteristics were not affected by any of the dietary treatments. The addition of 0.5, 1.0 and 2.0 kg/T CHL in feed decreased OTA accumulation in the liver by 13%, 34% ($P < 0.001$) and 38% ($P < 0.001$), respectively when compared with the diet contaminated with 90 µg/kg of OTA and that did not contain any CHL. These results demonstrated that CHL, when used as an adsorbent, could potentially reduce the deposition of OTA in the liver of broiler chicks. Increased inclusion of CHL in the diet could be correlated with a subsequent decrease in OTA accumulation in liver.

Key Words: mycotoxin, ochratoxin A, chicken, liver, mitigation

446P Effect of use of carvacrol and cinnamaldehyde in replacement of antibiotic growth promoters on the performance of broilers. G. Bosetti*, C. Facchi, L. Griebler, E. Aniecevski, G. Rossato, C. Baggio, and T. Petrolli, *Unoesc, Xanxerê, Brazil.*

The indiscriminate use of performance enhancing additives in broiler nutrition promotes increasing bacterial resistance and the risks of cross-resistance transmission to humans. Therefore, it is necessary to develop new alternatives to replace these additives, without compromising the proper development of the birds. Essential oils comprise a class of promising molecules that replace the growth promoters in broiler diets, as they exert an effect on intestinal pathogenic bacteria, as well as improve digestive capacity and antioxidant effect on the animal organism. The objective of this study was to evaluate the effect of the use of a blend of encapsulated essential oils in broiler feeding, in a 1–40 d-old period on performance (live weight, weight gain, feed intake, feed conversion and production efficiency index) of broiler chicks. The blend was composed of 60% of cinnamaldehyde, originating from cinnamon and 30% of carvacrol from oregano. A total of 600 male Ross broiler chickens were distributed in the first day of age in a completely randomized design, consisting by 5 treatments and 8 replicates, with 15 birds in each experimental unit, the means obtained were compared with each other by means of the 0,05 SNK variability test. The treatments were: T1 - negative control; T2-positive control (Virginamycin 15 ppm); T3 - 100 ppm essential oils; T4 - 200 ppm essential oils; T5 - 400 ppm essential oils. In all evaluations, birds fed the negative control diet presented lower body weight ($P < 0.05$), higher feed conversion ($P < 0.05$) and lower productive efficiency index ($P < 0.05$) when compared with the birds of the others tested treatments, which did not differ among them ($P > 0.05$), in all evaluated parameters. It was concluded that the use of carvacrol and cinnamaldehyde can adequately replace growth promoters without compromising poultry's performance.

Key Words: cinnamaldehyde, essential oils, poultry nutrition, promoters, resistance

447P Performance of broilers exposed to used litter and fed basal diets with Agolin Poultry, BMD/Stafac, or no additive from 0 to 42 days. M. D. Sims[1], B. Zweifel[2], P. Williams*[3], and D. M. Hooge[4], *[1]Virginia Diversified Research Corporation, Harrisonburg, VA, [2]Agolin S.A., Biere, Switzerland, [3]Advantec Associates Inc., Davis, CA, [4]Consulting Poultry Nutritionist, Eagle Mountain, UT.*

Agolin Poultry is a blend of encapsulated, essential oil compounds. A 42-d pen trial was conducted with 1,800 straight-run broiler chicks using 3 dietary treatments, 20 pens/treatment, and 30 chicks/pen to evaluate performance. A randomized complete block design was used, and if ANOVA was significant at $P \leq 0.05$, means were separated by Tukey's test ($P = 0.05$). Live coccidia vaccine was given at placement (d 0). Pelleted diets based on corn, soy, meat and bone, DDGS, and soy oil basal diets (CON) or basal diets with Agolin Poultry (200 mg/kg 0–14 d and 100 mg/kg 14–42 d) or BMD 55 mg/kg 0–28 d) and Stafac (22 mg/kg 28–42 d) were fed. Litter was new wood shavings initially with used litter added on d 4 to provide *Eimeria* and bacterial pathogens. Daily light:dark cycle was 16h:8h. Each pen had a bell-drinker and tube feeder. Litter was sampled at 14, 28, and 42 d for moisture and theoretical ammonia calculated from nitrogen. Oocysts per gram (OPG) feces were counted at 42 d. Footpad lesion severity scores (0–2) were taken at 42 d. The 28- and 35-d BW were heavier on Agolin (1.820 and 2.168 kg) or antibiotic diets (1.806 and 2.146 kg) than CON diets (1.190 and 1.749 kg) at $P = 0.005$ and 0.011, respectively. The BW gain from 14 to 28 ($P = 0.015$) or 14–35 ($P = 0.035$) days was greater on Agolin diets than CON with antibiotic diets intermediate. The 0–35 d mortality-adjusted feed conversion ratios (MAFCR) were lower on antibiotic (1.619) or Agolin (1.625) diets than CON diets. Mortality (2.46–3.18%) was unaffected by treatment. Ammonia tended to be lower for Agolin diets at 28 d. Litter moisture, OPG and footpad scores were not different among treatments. The 35-d BW and 0–35 d MAFCR were significantly improved by Agolin or antibiotic diets compared with CON diets.

Key Words: Agolin, antibiotic, broiler, essential oil, litter

448P Supplementing diets with a coated mix of betaine and antioxidants alleviates the detrimental effect of high temperatures on the growth performance of broilers. S. Toussaint, P. Moquet*, S. Klein, and N. Brévault, *MiXscience, Bruz, France.*

Energy metabolism of broilers is challenged when housing temperature exceeds thermo-neutrality. High housing temperatures increase energy maintenance requirements while inhibiting mitochondrial uncoupling proteins. These combined effects results in oxidative stress, impaired growth performance and altered carcass quality. Betaine is a trimethyl derivative of glycine having zwitterion and methyl donor properties. It acts, therefore, as an osmoprotectant. Feeding a combination of betaine and antioxidants may help birds to cope with summer temperatures. The use of such dietary strategy is, however, hampered by technological issues such as cacking. A specific coated mix of betaine and antioxidant (BeTaHit; B) was developed to prevent technological issues. The purpose of this trial was to evaluate the effect of BeTaHit on growth performance, oxidative stress markers and carcass quality of broilers subjected to high temperatures. The study was designed as a randomized complete block design with 3 groups: CTR (no BeTaHit), B750 (750 ppm B) and B1300 (1300 ppm B) in 16 blocks. Birds (n = 192) were kept under standard conditions from 1 till 20 d. High temperatures were applied every day from 21 till 35 d (28°C for 3 h; 30°C for 4 h; 28°C for 3 h; 24°C for 12 h). Growth performance was monitored throughout the trial. Plasmatic glutathione peroxidase (GHS), malondialdehyde (MDA) and creatine kinase (CK) activities were quantified for C and B1300 at 36 d. Hemolysis score, dripping loss and breast meat yield were measured across all treatment groups at d 36. Data was analyzed using one-way ANOVA. Differences were deemed significant at $P < 0.05$ and means were separated using Tukey's test. Birds fed B750 diets had significantly higher body weight gain ($P = 0.026$) and lower feed conversion ratio ($P = 0.021$) than their C and B1300 fed counterparts over the 0–35 d period. Birds fed C diets had significantly higher hemolysis score at d 36 than birds fed B750 diets ($P = 0.014$). Carcass quality, GHS, MDA, and CK were not significantly affected by dietary intervention ($P > 0.05$). A high dose of BeTaHit (B1300) did not have, therefore, any

significant effect on the measured oxidative stress markers while a lower dose (B750) improved significantly a cellular integrity marker. Hence, this study may suggest that BeTaHit supports the growth performance of birds subjected to high housing temperature by modulating cellular integrity rather than redox balance.

Key Words: coated betaine, housing conditions, growth peformance, carcass quality, oxidative stress

449P Humoral immunity of broiler chicks submitted to post-hatch fasting and supplementation of conjugated linoleic acid. P. Martins[1], L. Montel[1], A. Rabelo[1], A. Mesquita[2], J. Santos[1], L. Drosghic[3], and J. Stringhini*[1], *[1]Universidade Federal de Goias, Goiania, Goias, Brazil, [2]Instituto Federal Goiano, Rio Verde, Goias, Brazil, [3]Universidade do Estado de Mato Grosso, Nova Mutum, Mato Grosso, Brazil.*

In this experiment, we aimed to evaluate the humoral immunity of broiler chicks submitted to post-hatch fasting, and pre-starter diet supplemented with CLA. A total of 320 newly hatched chicks were housed in a completely randomized design, in a 2x2 factorial arrangement, considering fasting (0 or 12 h receiving only water and no feed) and CLA inclusion on the pre-starter diet (0 or 0.025%), totalizing 4 treatments and 8 replicates of 10 birds each. Diets to be provided at the first 12 h of life were crumbled, while diets for the other phases were mashed. After 12 h of fasting, immunoglobulin G (IgG) titers for Newcastle Disease were determined on blood serum and on yolk sac content. At 7 d old, and weekly up to 35 d old, IgG titers were determined on blood serum. At all of the evaluated ages, IgG titers were determined by ELISA assay, with the help of Biochek software. Data were submitted to ANOVA ($P < 0.05$), using R software. After 12 h, chicks submitted to post-hatch fasting showed lower IgG titers in the yolk sac ($P = 0.008$), but higher titers on the blood serum ($P = 0.004$). CLA supplementation reduced IgG titers in the yolk sac ($P = 0.007$), but led to higher titers on the blood serum ($P = 0.018$). There was an interaction of the factors for the blood serum titers ($P = 0.028$). Lower IgG titers were found in the serum of non-supplemented chicks which were not submitted to fasting. At 7 d old, there was no isolated effect of the supplementation ($P = 0.392$) or fasting ($P = 0.135$) on the humoral immunity of chicks. The same results were found at 14 d old ($P = 0.943$ and $P = 0.206$); at 21 d old ($P = 0.926$ and $P = 0.258$) and at 35 d old ($P = 0.181$ and $P = 0.070$, for the post-hatch fasting and for the CLA supplementation, respectively). Although, at 21 d old, there was an interaction of the factors ($P = 0.010$). Fasting increased IgG titers in the non-supplemented chicks. At 28 d old, fasting reduced immunoglobulin titers on the blood serum ($P = 0.002$). In conclusion, post-hatch fasting and CLA supplementation accelerate the transfer of immunoglobulins from the yolk sac content to the blood serum.

Key Words: immunoglobulin, newly hatched chick nutrition, omega-6, pre-starter diet, yolk sac

450P Effect of plant extract derived from *Minthostachys mollis* on performance of broiler chicks at 21 days of age. E. Salvador*, *National University "San Luis Gonzaga" of Ica-Perú, Chincha, Peru.*

A study was carried out to evaluate the effect of plant extract in drinking water derived from *Minthostachys mollis* (muña) on the productive performance of chicks from 0 to 21 d of age, and to determine whether it could have the potential as alternative natural growth promoter to antibiotics. A total of 192, 1 d - old male BB broilers chicks Cobb 500 were randomly assigned to 3 experimental groups as treatments and 4 replicates each: 1. Basal diet, without antibiotic; 2. Basal diet + 50 mg Zinc Bacitracin / kg; 3. Basal diet + *Minthostachys mollis extract*, in a randomized complete block design. We evaluated live weight, weight gain, feed intake, feed conversion, energy efficiency and margin over feed cost. The data were analyzed using the GLM procedure of SAS v.9 (2002) followed by Tukey's mean test. The results showed that supplementation with *Minthostachys mollis* extract significantly increased ($P = 0.05$) feed intake of chicks supplemented with vegetable extract (1291 g) compared with the group with antibiotics (1266 g). Live weight, weight gain, feed conversion and energy efficiency were not affected ($P > 0.05$); However, the group of chicks fed the plant extract achieved a weight gain of 932.75 g, which represents a numerical improvement of 27.8 g (3.07%) compared with the group with antibiotics (904.95 g). The feed cost for ton was of $ 555.74 for diet with antibiotic and $ 556.32 for diet with extract. The margin on feed cost was better for the group of chicks with the plant extract about of 8.14%. It was concluded that the plant extract of *Minthostachys mollis* increases feed consumption, maintains productive performance and improves profitability, and can be considered as a potential natural growth promoter to replace antibiotics in the chicks diet in the 0 to 21 d of age.

Key Words: chick, plant extract, antibiotic, performance, *Minthostachys mollis*

451P Association of symbiotic and nutritive gel in neonate chicks submitted to fasting before allotment in organs morphometry. F. Castejon[1], E. Oliveira[1], J. Martins[1], I. Araujo[2], M. Andrade[1], and J. Stringhini*[1], *[1]Universidade Federal de Goias, Goiania, Goias, Brazil, [2]Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.*

In this study, a synbiotic (PoultryStar sol) associated with a nutritive gel in the hatchery on broiler performance, digestibility, blood biochemical parameters, organs morphometry and carcass yield were evaluated. 400 male neonate Cobb 500 chicks were fed in the hatchery and then transported to experimental facilities and allotted in battery cages, in 5 treatments and 8 replicates of 10 birds each. Treatments were: CN- negative control; G – gel in hatchery; GS – Gel plus symbiotic in hatchery; GSS – Gel plus symbiotic in the hatchery and symbiotic, in alternated days, in water; S – Symbiotic in water in alternated days. Symbiotic was offered during the 3 d after allotment; before, on the first day and a day after diet change and once a week between treatments; so GSS received the symbiotic in the water and d 2, 3, 7, 10, 11, 12, 14, 22, 23, 24, 28 (12 time points) and S at d 2,3,4,7,10,11, 12, 14, 22, 23, 24, 28 (12 times points). All groups remained in a 24 h period of fasting of feed and water, after hatchery expedition. The live weight, total intestinal length and segment measurements, relative weight of intestine, pancreas, proventriculus and gizzard, liver and heart were evaluated at 12, 24 and 42 d of age. Statistical analysis was performed using ANOVA and the Scott-Knott test applied for comparison of the means using the R Software. There were no differences in total intestinal length or segments, but the relative weight of some organs differed between treatments. Pancreas at 12 d of age was increased in Group G, but the effect didn't persisted in older ages. Bursa relative weight were affected in 24 and 42 d of age, with higher values for the control group at 24 d and the opposite in 42 d of age. The group S had similar values to control group. Spleen were increased for control group at 24 d of age, but no effect occurred in other ages. These findings can suggest a possible effect in the humoral immune response. The early supplementation of nutritive gel associated or not to the symbiotic can stimulate the functional development of the gastrointestinal tract.

Key Words: additive, broiler, microbiota, nutritive gel, synbiotic

452P *Bacillus subtilis* DSM 32315 alone or combined with butyric acid improves performance of broiler chickens. J. C. D. P. Dorigam*[1], K. Doranalli[1], and S. Delgado[2], *[1]Evonik Nutrition & Care GmbH, Hanau, Germany, [2]Evonik España y Portugal S.A., Granollers, Spain.*

Direct-fed microbial (DFM) are used in poultry diets to control growth of pathogenic bacteria allowing the development of healthier birds. In addition, Butyric acid has been supplemented in poultry for better development of epithelial cells and maintenance of mucosal integrity. The combination of both products may improve the nutrient utilization and maximize performance. Thus, the aim of this trial was to evaluate the effects of *Bacillus subtilis* DSM 32315 alone or in combination with calcium butyrate on broiler's performance. A total of 240 Cobb 500 male day-old chicks were randomly assigned to 4 treatments with 6 replicates of 10 birds/pen. Corn-soybean meal basal diets were formulated to meet nutritional recommendations for starter (1–14 d), grower (15–28 d) and finisher (29–43 d) phases. The treatments were: 1) Control (no additive), 2) Control + calcium butyrate, 3) Control + *B. subtilis* DSM 32315 (DFM), and 4) Control + calcium butyrate + *B. subtilis* DSM 32315. Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were recorded. Two birds/pen were sacrificed at 21 and 28 d to collect digesta from ileum and cecum for bacterial count. Data was analyzed by one-way ANOVA followed by multiple comparisons of means using the Student-Newman-Keuls test and a p-value <0.05 was deemed significant. Feeding butyrate or DFM alone did not significantly improve FCR or BWG, however birds fed DFM had 1% higher BW and 1% lower FCR compared with the control. Most interestingly, FCR was significantly improved ($P < 0.05$) with the combination of DFM and Butyrate (1.557 vs. 1.645 g/g), compared with the control group. In addition, only *C. perfringens* counts in the cecum was significantly ($P < 0.05$) reduced by feeding Butyrate (16.7%) and DFM (26.4%) alone or in combination (34.7%), at 21 d. Furthermore, the European efficiency factor was numerically higher in birds fed DFM and a combination of DFM and Butyrate compared with the control or only Butyrate fed birds. In conclusion, results of the study support the hypothesis that *B. subtilis* DSM 32315 alone or in combination with calcium butyrate were effective to reduce the population of *C. perfringens* in the intestine and improve feed conversion of broiler chickens allowing for higher economical returns.

Key Words: broiler, *Clostridium perfringens*, direct-fed microbial, organic acid, performance

453P A live performance and carcass evaluation of broilers fed diets supplemented with phytogenic XTRACT 6930 (microencapsulated) or with different antibiotic growth promoters BMD or Stafac. J. Maurin[1], M. D. Sims[3], and D. M. Hooge*[2], *[1]Pancosma S.A., Geneva, Switzerland, [2]Consulting poultry nutritionist, Eagle Mountain, UT, [3]Virginia Diversified Research Corporation, Harrisonburg, VA.*

A standardized micro-encapsulated blend of carvacrol, cinnamaldehyde, and capsicum oleoresin (XTRACT 6930, XT6930) is known to improve digestibility, to maintain gut architecture, and to modulate inflammation consequently supporting live performance in broilers. A 42-d pen trial was conducted with 1,200 straight-run Cobb 500 chicks using 4 dietary treatments, 10 pens/treatment, and 30 chicks/pen (1.22 × 1.52 m) to evaluate performance. A randomized complete block design ($P \leq 0.05$) was used with Tukey's test ($P = 0.05$) to separate means. Lighting was continuous for 24-h daily. Live coccidia vaccine was given at placement (d 0) and used litter was applied to each pen on d 4 as a mild disease challenge frequently occurring in broilers operations. Economical, low protein pelleted diets (0–14, 15–28, 29–42 d) based on corn, soybean meal, meat and bone, DDGS, and soybean oil were fed. Treatments were: basal diets (Control, CON) or basal diets + XT6930 at 90 g/short ton, BMD (bacitracin) at 50 g/short ton, or Stafac (virginiamycin) at 20 g/short ton. Live performance was measured at 14, 28, and 42 d. On d 43, 2 males and 2 females per pen weighing close to pen mean for each sex were processed (after 10 h feed and 1 h water fast) for dry carcass and bone-in breast yields and moisture (drip) loss at 30°C for 48 h. The XT6930 had highest BW at each age, significantly greater than BMD at 14 d and than CON at 42 d, with other groups intermediate in each case. The XT6930 had significantly lower feed conversion value (FCV) or mortality-adjusted FCV (MAFCV) at 14 d than CON, and the additive treatments tended lower in FCV ($P = 0.063$) and MAFCV ($P = 0.109$) than CON at 42 d. Mortality % were 11.3, 7.67, 7.33, and 7.67%, respectively, from 0 to 42 d ($P = 0.194$). Sampled bird BW were 1.947^{c}, 2.091^{b}, 2.003^{bc}, and 2.039^{ab} kg with dry carcass % of BW at 75.15^{b}, 75.70^{ab}, 76.97^{a}, and 76.65^{ab}, respectively. Dry bone-in breast weights were 389^{c}, 459^{a}, 412^{bc}, and 433^{ab} g with yield % of BW at 19.99^{b}, 21.95^{a}, 20.59^{b}, and 21.26^{ab}, respectively. Bone-in breast moisture loss % were 22.78^{ab}, 19.29^{b}, 21.09^{ab}, and 24.47^{a}, respectively. The XT6930 diets improved BW *vs*. CON or BMD diets, FCV and MAFCV *vs*. CON diets, increased bone-in breast weight and yield *vs*. CON or BMD diets, and reduced moisture loss % *vs*. Stafac diets.

Key Words: antibiotic, breast, broiler, carcass, phytogenic

454P Chicken as the new omega-3 source: DHA enrichment of broiler tissues after 21 or 42 days of dietary supplementation with dried microalgae powder (All-G-Rich). M. Morlacchini[1], G. Fusconi[1], T. Ao*[3], J. Keegan[2], and C. Moran[2], *[1]CERZOO S.r.l, Piacneza, Italy, [2]Alltech SARL, Vire, France, [3]Alltech Inc., Nicholasville, KY.*

The omega-3 fatty acid (n-3 FA) content of chicken meat can be altered by adding n-3 FA rich ingredients to chicken feed, increasing the nutritional value of poultry products. The docosahexaenoic acid (DHA)-rich microalgae *Aurantiochytrium limacinum* (AURA; CCAP 4087/2; All-G-Rich, Alltech Inc., KY, USA) can be heterotrophically grown in a sustainable manner and fed to chickens to increase tissue n-3 FA content. The objective of this study was to evaluate n-3 FA enrichment of broiler tissues following 21 or 42 d supplementation with AURA. The study was conducted using 350 male ROSS 308 chickens, with 10 animals per pen. Pens were randomly assigned to 1 of 5 treatments: Unsupplemented control (T1); AURA provided from d 0–42 at a rate of 0.5% (T2) or 1% (T3) of the diet; AURA provided from d 21–42 at a rate of 0.5% (T4) or 1% (T5) of the diet. Treatments were provided for 42 d after which one bird per pen was sacrificed and DHA content of breast, thigh, liver, kidney and skin (with fat) was determined. The DHA content of breast tissue increased with each level of supplementation ($P = 0.002$). However, providing the same dose for twice as long did not result in higher levels of breast enrichment, with no differences observed ($P > 0.05$) between T2 and T4 (32.6 vs 30.3 mg DHA /100g tissue) or T3 and T5 (55.2 vs 50.1 mg DHA /100g tissue). The same trend was observed in thigh muscle, with dose rate increasing the level of enrichment observed ($P < 0.05$), but no differences between the level of enrichment when the same dose level was provided for 21 or 42 d (53.6 vs 46.6 mg DHA /100g tissue for T2 and T4; 86.0 vs 79.6 mg DHA /100g tissue for T3 and T5). A higher efficiency of DHA transfer from diet into the breast (6, 4.7, 7, and 5.9% for T2, T3, T4 and T5, respectively) and thigh (9.2, 7.4, 10.8, and 8.3% for T2, T3, T4 and T5 respectively) tissues was observed when the diets were provided for

the shorter period of 21 d. For skin, liver and kidney, supplementation increased DHA content compared with the control in all cases, however differences between treatments were more variable than for breast and thigh tissue. These results indicate that dietary supplementation for 21 d before slaughter increases tissue DHA content and has a higher efficiency of DHA transfer from feed to tissue, with no added benefit if fed for the entire 42 d program.

Key Words: algae, broiler, DHA enrichment

455P Effects of a specific blend of sensory molecules from essential oils and oleoresins of spices compared to an antibiotic growth promoter program on the broiler feed efficiency. J.-F. Gabarrou*[1], A. Wagner-Wells[2], B. Medina[2], and I. Girard[2], *[1]Phodé Sciences, TERSAC, France, [2]Probiotec International, Sainte-Hyacinthe, Canada.*

Antibiotic use as growth promotors in animal feed is highly regulated, banned, or limited due to consumer demand. Phytogenics have been shown and are being used as alternatives to antibiotics. Oleobiotec Poultry (OLEO) is a sensory feed additive (PHODE, Albi, France) comprised of an essential oil blend (mainly Oregano) and spices (mainly pipperin and capsaicin) that was developed to impact growth performance and feed efficiency. The objective of this study was to determine performance and nutrient efficiency of broilers under a practical challenge. One day old Ross 308 were placed into 6 pens × 3 treatments (18 pens × 24 birds). The positive control (T1) was formulated to conform to Ross specifications with 55 ppm of Bacitracin Methylene Disalicylate (BMD). Treatments 2 (T2) and 3 (T3) were fed a low nutrient diet, where metabolizable energy, crude protein and amino acids were reduced by 2.5%. T2 diet included BMD (55 ppm), and T3 included OLEO (100 ppm). A sanitary challenge was simulated by an anticoccidian vaccination program using 5 times the normal dose. An extra dose was also sprayed on the chick's litter. Feed intake (FI), live BW and feed conversion ratio (FCR) were estimated at 37 d. Nitrogen and Phosphorus losses were calculated according to body composition provided by Ross and using the European simplified balance method. The N and P conversion ratios were calculated by the total N or P intake and divided by the total N or P fixed in the body. Statistical analysis was carried out, for all indicators, using a one way ANOVA with treatment as the fixed factor in the mixed procedure of SAS software (SAS v. 9.3 Cary, N.C.). There was no significant effect ($P = 0.231$; T1: 4447 ÷ 29g; T2: 4417 ÷ 25G; T3: 4398 ÷ 23g) of treatment on FI. Live BW was significantly lower ($P < 0.001$) for T2 (2484 ± 32g) than T1 (2568 ± 22g) and T3 (2552 ± 21g). However, there was no significant effect ($P = 0.146$; T1: 1.740 ± 0.062; T2:1.774 ± 0.076; T3: 1.718 ± 0.052) of treatment on FCR. The N conversion ratio was significantly reduced ($P < 0.05$) in T3 (1.562 ± 0.032) compared with T1 (1.609 ± 0.044) and T2 (1.615 ± 0.056). The P conversion ratio was significantly reduced ($P < 0.05$) in T3 (1.517 ± 0.031) compared with T1 (1.581 ± 0.042) and T2 (1.567 ± 0.055. The sensory feed additive (OLEO) is a good growth promotors alternative. It could improve broilers feed efficiency and reduce feed cost by reducing amino acids and phosphorus specification of the feed.

Key Words: phytogenic, functional sensory molecule, essential oil, broiler, feed efficiency

456P Dietary spray-dried plasma influences early intestinal development and broiler chick growth. Y. Fasina*[1], P. Ferket[2], C. Blue[1], Y. Jababu[1], A. Hooks[1], and R. Noble[1], *[1]North Carolina A&T State University, Greensboro, NC, [2]North Carolina State University, Raleigh, NC.*

The continued increase in consumer demand for antibiotic-free poultry meat necessitates continuous search for alternative growth-promoting products. Spray-dried plasma (SDP) contain growth factors and functional proteins (such as albumin), and may therefore have potential to replace antibiotics in broiler diets. An experiment was conducted to evaluate the effect of porcine SDP supplementation on early intestinal development and growth performance of broiler chicks during the first 2 weeks of life. Day-old (240) Ross 708 male chicks were obtained from a commercial hatchery, weighed, and randomly assigned to 6 dietary treatments. Treatment 1 (CX) consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without SDP. Treatment 2 (MX) consisted of chicks given unmedicated corn-SBM basal into which Bacitracin methylene disalicylate (BMD) was added at 0.055g/kg diet. Treatments 3 (SP1), 4 (SP2), 5 (SP3), and 6 (SP4) consisted of chicks given unmedicated corn-SBM basal into SDP was added at 10, 20, 30, and 40 g/kg diet, respectively. Each treatment consisted of 4 replicate pens, with each pen housing 10 chicks. Body weight (BW), body weight gain (BWG), feed intake, and feed conversion ratio (FCR) was monitored over a 2-week period. To assess early intestinal development, tissue segments were collected on d 7 of experiment from the jejunum and analyzed for alkaline phosphatase (ALP) activity (µmol/min/µg protein). Data was analyzed using one-way ANOVA. Results showed that MX and SDP treatments had similar ALP activity ($P > 0.05$). At the end of the experiment (d 14), there were no differences in BW and BWG among treatments ($P > 0.05$). However, SP3 (1.15) and SP4 (1.17) had superior FCR ($P < 0.05$) compared with other treatments (1.22 to 1.27). It was concluded that dietary supplementation of SDP at 30 g/kg diet had similar effect as MX treatment on intestinal development and chick growth during the first 2 weeks of life. SDP may be able to replace antibiotics such as BMD in broiler chick diets.

Key Words: spray-dried plasma, bacitracin methylene disalicylate, alkaline phosphatase, chick growth performance

457P The effect of supplementary guanidinoacetic acid on metabolisable energy in broiler diets. S. P. Rose and V. Pirgozliev*, *Harper Adams University, Newport, United Kingdom.*

This study investigated the effect of guanidinoacetic acid (GAA) on dietary apparent metabolisable energy (AME), total-tract dry matter (DMR), nitrogen (NR) and fat retention (FR) coefficients when fed to male and female Ross 308 chicks. Three dietary treatments were used; a negative control (NC; −50 kcal AME/kg); a positive control (PC; recommended AME with levels of 3000 and 3100 kcal/kg in starter and grower diets); third diet NC + 0.06% GAA. Each dietary treatment was fed to 16 single sex replicates (8 female; 8 male) with 20 birds, following randomization. At 20 d of age, 2 chickens from each pen with a BW nearest to the pen average were transferred to 1 of 48 metabolism cages following the same randomization and dietary treatments as in the floor pen phase. Feed and water were offered ad libitum. The chickens were kept in the cages for approximately 96 h until 24 d of age, and total excreta output were collected in the trays beneath. Feed intake for the same period was recorded for the determination of dietary AME, DMR, NR and FR. A randomized block ANOVA was performed and a 2 × 3 factorial structure was used to investigate the main treatment factors (sex × diets) and their interaction. There were no differences ($P > 0.05$) in AME (13.54, 13.36, 13.56 MJ/kg DM; SEM = 0.199), DMR (0.724, 0.721 0.734; SEM = 0.0133) and FR (0.809, 0.839, 0.843; SEM = 0.0219) between the experimental diets in the study, respectively. However, the NR coefficients for NC was lower ($P < 0.05$; SEM = 0.0315) compared with PC and NC+0.06% GAA, and was 0.540, vs 0.649 and 0.685, respectively. There were no differences between the 2 sexes used

in the experiment and no sex by diet interactions were observed ($P > 0.05$). Results of this study indicate that an addition of 0.06% GAA to the diet might improve net protein utilization of poultry feed.

Key Words: guanidinoacetic acid (GAA), metabolisable energy, digestibility, broilers

458P Effects of star anise (*Illicium verum* Hook. f.) essential oil on laying performance and antioxidant status of laying hens. C. Yu*[1], C. Yang[2], Z. Yang[1], and G. Li[3], *[1]Shandong Agricultral University, Taian, Shandong, China, [2]University of Manitoba, Winnipeg, MB, Canada, [3]LinYi BLJ Food Co., Ltd., Liyi, Shandong, China.*

To investigate the effects of dietary supplementation of star anise oil on performance and antioxidant status of laying hens, 864 Hy-Line brown laying hens at 26 wk of age were randomly allocated to 4 treatments with 6 replicates of 36 birds. Dietary treatments were non-star anise oil (SAO) supplementation and supplemented with SAO at the level of 200, 400, 600 mg/kg diet. The birds were fed the diets for 56 d. Average egg weight, average daily feed intake, egg mass, laying rate and feed conversion of each replicate were measured. Blood and liver samples from 12 birds were obtained, 72 eggs were picked out, per treatment at d28 and d56 of the experiment, and eggs stored for 56 d, to determine antioxidant status in serum, liver and yolk. All laying hens had similar ADFI, average egg weight, egg mass, laying rate and feed conversion in d29 to d56 or the entire period of the experiment. However, increasing diet concentration of SAO tended to improve ($P < 0.10$) egg mass, average egg weight and ADFI in d1 to d28. Supplementation of SAO linearly increased ($P < 0.05$) activities of total superoxide dismutase (TSOD) (d28 and d56) and glutathione peroxidase (GSHPx) (d56) in serum, GSHPx (d28 and d56) in liver and total antioxidant capacity (T-AOC) (d56) in serum and liver, but linearly reduced ($P < 0.05$) concentrations of malondialdehyde (MDA) (d28 and d56) in liver. Supplementation of SAO linearly increased ($P < 0.05$) T-SOD activity at d14 and d28, reduced ($P < 0.05$) MDA concentration at d42 and d56 of the experiment in yolk. Increasing content of SAO linearly ($P < 0.05$) increased T-SOD activity in yolk of eggs stored at d 0, 14, 28, 42 and 56, decreased MDA content of eggs stored at d42 and 56, whether laying hens fed diets for 28 or 56 d. Dietary supplementation of SAO enhanced laying performance and overall antioxidant status of laying hens in a dose-dependent manner.

Key Words: star anise oil, laying hen, laying performance, antioxidant status

459P Effects of essential oil on laying performance, antioxidant status and egg quality of laying hens. Z. Yang*[1], G. Li[2], C. Yang[3], and X. Zhou[1], *[1]Shandong Agricultral University, Taian, Shandong, China, [2]LinYi BLJ Food Co., Ltd., Linyi, Shandong, China, [3]University of Manitoba, Winnipeg, MB, Canada.*

To investigate the effects of essential oil (contained cinnamaldehyde 60 g/kg, Garlic meal 800 g/kg and Maltodextrin140 mg/kg) on laying performance, antioxidant status, egg quality and DHA of laying hens. Six thousand Hy-Line brown laying hens at 65wk of age were randomly allocated into 2000 cages that were then randomly divided into 2 dietary treatments (1000 cages per treatment, 250 cages per replicates, 3birds/cage). The 2 treatments (TRT) were: 1) Control, laying hens were fed corn-soybean meal diets; 2) TRT 1 + 600 mg/kg essential oil. All laying hens had similar laying rate, ADFI, egg shape index, eggshell thickness, egg yolk color, haugh units and egg yolk weight. However, as compared with those of control treatment, laying hens supplemented with essential oil increased ($P < 0.05$) average egg weight and egg mass, decreased ($P < 0.05$) feed conversion (g:g). Supplementation of essential oil increased ($P < 0.05$) the activity of SOD, GSH-PX and T-AOC in serum, increased ($P < 0.05$) the activity of T-AOC in egg yolk, and decreased ($P < 0.05$) MDA content of laying hens in serum and egg yolk than the control group. There were higher ($P < 0.05$) DHA content in egg yolk and in liver with laying hens diet supplemented essential oil. Dietary supplementation of essential oil could improve laying performance, serum and liver antioxidant status, and DHA content in egg yolk and liver of laying hens.

Key Words: cinnamaldehyde, garlic meal, laying hen, laying performance, antioxidant status

460P Use of the probiotic *Bacillus subtilis* in broilers feeding at different ages. P. G. Urayama*[1], E. J. Nishiyamamoto[2], J. Pia[2], R. G. A. C. Araujo[1], L. C. Lopes[2], N. S. Oliveira[2], S. Gerhards[2], J. Cruvinel[2], M. de Andrade[2], B. Schmitz[2], E. C. Weiss[2], E. Muro[3], and S. E. Takashi[2], *[1]Universidade Estadual Paulista, Botucatu, Brazil, [2]Universidade Tecnológica Federal do Paraná, Dois Vizinhos, Brazil, [3]UNESP-São Paulo State University, Botucatu, Brazil.*

Probiotics are supplements containing live microorganisms that will act on the intestinal flora of the host and influence beneficially the health of this animal through the balance of the microbiota. This substance has been observed with an effective alternative to replace growth promoting antibiotics. The objective of this study was to evaluate the addition of probiotic *Bacillus subtilis* in broiler rations of different ages (to 14-d old and 28-d old). A total of 2 hundred and 24 male Cobb chickens were placed in 1m2 pens with reused wood-shaving litter. The experimental design was completely randomized with 4 treatments: NC (negative control, without antibiotic inclusion); PC (positive control, with zinc bacitracin inclusion); *Bacillus subtilis* inclusion (0,01%) of 1 to 14-d old and *Bacillus subtilis* inclusion (0,01%) of 15 to 28-d old, and 4 replication of 14 birds per pen. Diets were isoenergetic and isoprotein. The diets used were formulated with base of corn and soybean meal provided *ad libitum* during the experimental period. Birds and experimental diets were weighed at 28-d old to determine feed intake (obtained through the difference between total feed provided and collected at the end of each period), feed conversion (calculated as the ratio between total feed intake and weight gain and corrected by the weight of dead birds), and weight gain. At 28-d old the feed intake was less and consequently better feed conversion ($P < 0,05$) for treatments with *Bacillus subtilis* inclusion of 1 to 14-d old and 15 to 28-d old. Antibiotics were not efficient because it is an experimental shed free of pathogenic microorganisms Thus, the results of this study indicate that the addition of *Bacillus subtilis* (0,01%) can be used to improve performance both during the 1 to 14-d old as in the period of 15 to 28-d old.

Key Words: antibiotic, additive, performance

461P GC-FID method validation of DHA in layer hen feed supplemented with heterotrophically grown, dried microalgae powder (All-G-Rich). G. Dillon[3], W. Brandl[1], C. Cardinall[1], W. Yuan[1], T. Ao*[2], A. Yiannikouris[2], and C. Moran[3], *[1]Mérieux Nutrisciences, Burnaby, BC, Canada, [2]Alltech Inc., Nicholasville, KY, [3]Alltech SARL, Vire, France.*

A gas chromatography-flame ionization detection (GC-FID) method was validated and verified to demonstrate the suitability of the method in analyzing DHA in layer hen feed supplemented with dried microalgae powder. The characteristics studied during the validation included;

linearity, accuracy, reproducibility, LOD and LOQ and specificity. Commercial reference standards (NIST SRM 3275–2 Anchovy Oil and NIST 3290- Dry Cat Food) were also analyzed to demonstrate the suitability of the method. The sample preparation involved freeze drying control (blank) layer hen feed samples before mixing samples with dried microalgae powder (All-G Rich; Unextracted *Aurantiochytrium limacinum* biomass, Alltech Inc. KY, USA) before extracting the DHA analyte which was analyzed using GC-FID instrumentation. The method was successfully validated over a linear range of 0.3 mg/ml to 15 mg/ml. Accuracy experiments were conducted and the recoveries were within the acceptable limits of 90 – 110%. Reproducibility samples spiked with 0.5% and 1.0% dried microalgae powder were within acceptable limits of 10% RSD. The LOD and LOQ were established as 0.001% and 0.004% respectively. The robustness of the method was confirmed by comparing data sets from 2 different analysts establishing that validation parameters were all within acceptable limits. To assess the specificity, blank layer hen feed samples were analyzed in triplicate to confirm that the blanks contained no endogenous DHA and spiking studies showed DHA could be differentiated from other substances present in the blank feed. In conclusion, the method has been deemed to be fit for purpose in measuring concentrations of DHA in layer hen feed supplemented with dried algae powder rich in DHA for regulatory and efficacy studies.

Key Words: DHA, layer egg, algae, validation, feed

462P Effect of an algae-clay mix on the use by broiler chickens of a diet containing corn DDGS. M. Gallissot*, M. G. Suarez, and M. A. Rodriguez, *Olmix, Brehan, France.*

This study was set up to evaluate the effect of supplementing an algae-clay mix (MFeed+) on growth performance of broiler chickens fed with corn DDGS. Four-hundred-and-14 1-d-old chicks were randomly distributed to 18 pens, allocated to 1 of 3 groups receiving different diets: the standard diet (C-), the test diet (T-), containing corn DDGS at the level of 10%, and the test diet supplemented with 0.1% of algae-clay mix (T+). Three different feeds were distributed from D0-D9, D9-D18 and D18-D31. Group weighing of the animals (D0, D9, D18 and D31) and litter quality scoring (D18) were performed. Results were submitted to ANOVA. Results show a significant decrease of ADWG in the finishing period (−8.61%, $P = 0.04$) and the total period (−7%, $P = 0.02$) in the T- group compared with the control. In finishing and total periods, the ADWG of the T+ group is significantly higher than in the T- group (respectively +11.72%, $P = 0.02$ and +7.13%, $P = 0.03$) and is similar to the control. On the other hand, in the starter period, C- and T- groups show a significantly higher ADWG than the T+ group. Mortality was non-significantly lower in the T+ group than in C- and T- groups. No visible impact was observed on litter quality. In the end, this study shows a positive effect of the algae-clay mix on growth performance of broiler chickens fed with the test diet, raising the interest of its use in the utilization of such diets.

Key Words: corn DDGS, algae, clay

463P Feeding high-oleic peanuts to layer hens enhances egg yolk color in shell eggs. O. Toomer*[1], T. Vu[1], A. Hulse-Kemp[1], L. Dean[1], R. Malheiros[2], and K. Anderson[2], *[1]US Dept. of Agriculture-Agricultural Research Service, Raleigh, NC, [2]North Carolina State University, Raleigh, NC.*

Previous studies have identified normal-oleic peanuts as a suitable and economical broiler feed ingredient. However, no studies to date have examined the use of high-oleic (HO) peanut cultivars as a feed ingredient for poultry and determined the impact of feeding HO peanuts on poultry performance, nutritive or sensory qualities of the eggs or meat produced. This project aimed to examine the use of HO peanuts, as a feed ingredient for layer hens to determine the effect on performance, egg lipid chemistry and quality of the eggs produced. Forty-eight 40-week-old layer hens were fed a conventional soybean meal + corn control diet or a HO peanut + corn diet for 10 weeks in conventional battery cages. Body and feed weights were collected weekly. Pooled egg samples were analyzed for quality, lipid analysis and peanut protein allergenicity. There were no significant differences in hen bodyweight, feed intake, number of eggs produced, USDA grade quality, egg albumen height or egg haugh unit between the treatment groups. Eggs produced from layer hens fed the HO peanut diet had significantly ($P < 0.05$) greater yolk color scores (2-fold), HO fatty acid and β-carotene levels in comparison to the controls. Eggs produced from layer hens fed the control diet had significantly greater content of saturated fatty acids and trans-fat in comparison to eggs produced from layer hens fed the HO peanut diet. All egg protein extracts from both treatments at each time point were non-reactive with rabbit Anti-Peanut Agglutinin antibodies. This study identifies HO peanuts as an abundant commodity that could be used to support local agricultural markets of peanuts and poultry within the southeastern US and be of economic advantage to producers while providing a potential health benefit to the consumer with improved egg nutrition.

Key Words: high-oleic peanuts, feed ingredients, layer hens, shell eggs, egg yolk

464P Effect of dietary supplementation of *Bacillus subtilis* 29784 on performance and egg quality in laying hens from week 19 to 48 of age. M. Neijat*[1], R. Shirley[2], J. Barton[2], P. Thiery[3], A. Welsher[2], and E. Kiarie[1], *[1]University of Guelph, Guelph, ON, Canada, [2]Adisseo USA Inc., Alpharetta, GA, [3]Adisseo France SAS, Antony, France.*

The aim of the current study was to evaluate the performance and egg quality in laying hens over a 30-week period when fed Alterion-NE50, a single strain of *Bacillus subtilis* (SSB; DSM29784, Adisseo, USA). The 4 dose-response treatments consisted of a nutritionally adequate, corn-soybean meal base diet that contained either no probiotic (control, CON), 1.1E+08 (low, LSSB), 2.2E+08 (medium, MSSB) or 1.1E+09 (high, HSSB) cfu/kg of diet. A total of 336 Shaver White layers were used, with 12 replicate cages/treatment. In the Layer I phase (week19 to 28), stocking density was maintained at 7 birds/replicate. In the Layer II phase (wk 29 to 48), stocking density was maintained at 6 birds/replicate. Feed intake (FI), body weight (BW), feed conversion ratio (FCR; g FI/ g egg mass), and the following egg parameters were evaluated: egg production (EP), egg weight (EW), egg mass (EM), and egg quality indices, shell thickness (ST), shell breaking strength (SBS), albumen height (AH), Haugh unit (HU), yolk color (YC) and individual egg component weights and microbial load (total aerobic bacteria). Data obtained on a biweekly or monthly basis, were analyzed as a one-way ANOVA using PROC GLM in SAS and adjusted using Tukey's test to separate means, with significance defined at $P < 0.05$. Overall, FI and BW were not influenced by SSB supplementation in either phase; however, in the early Layer II period (wk 32), BW increased in a linear response ($P = 0.019$) to SSB inclusion, possibly reflecting a better adaptation to diet change. In Layer I, supplementing SSB at 1.1E+08 cfu/kg feed increased EM (g/egg) compared with control by 3.3% ($P = 0.049$), EW (g/egg, a trend $P < 0.066$) by 1.2%, and EP by 2% (a quadratic trend, $P = 0.057$). In Layer II, a dose response to SSB inclusion improved FCR (linear trend, $P = 0.094$; g FI: g EM). There was no diet

effect on ST; however, SBS was lowest at wk 20 with the highest SSB dose (4.518 vs. 4.889 kgf for HSSB vs. CON; $P = 0.045$). The AH and HU were improved at higher doses of SSB, in both the Layer I and II phases ($P \leq 0.005$). No significant treatment effects were noted in egg component weights (egg yolk, shell and albumin), shell index, or the total microbial count in the egg components. In summary, while the inclusion of 1.1E+08 cfu/kg feed of *B. subtilis* 29784 improved hen performance and maintained egg quality parameters in both Layer I and II phases, a higher dose of SSB improved the interior protein quality of the egg (AH and HU indices).

Key Words: *Bacillus subtilis*, laying hen, hen performance, egg quality

465P Making chicken meat healthful again: DHA enrichment following dietary supplementation of heterotrophically grown, dried microalgae powder (All-G-Rich). C. Moran[2], D. Currie[3], A. Knox[3], J. Keegan[2], and T. Ao*[1], *[1]Alltech Inc., Nicholasville, KY, [2]Alltech SARL, Vire, France, [3]Roslin Nutrition, Aberlady, United Kingdom.*

Omega-3 fatty acid (n-3 FA) rich microalgae can be heterotrophically produced in a sustainable manner and added to chicken diets to increase the n-3 FA content of chicken meat and eggs, improving their nutritional value. The aim of this study was to investigate the effect of dietary supplementation of broilers with a docosahexaenoic acid (DHA) rich microalgae (*Aurantiochytrium limacinum*, (AURA) CCAP 4087/2, All-G-Rich, Alltech Inc.) on productivity and meat n-3 FA content. Healthy day-old male Ross 308 chicks (n = 640) were randomly allocated to one of 4 diets which were provided ad libitum. All birds were fed a starter diet from d 0–21. The experimental diets contained AURA at a rate of 0%, 0.25%, 0.5% and 1% and were provided from d 22–42. The study was conducted using 64 pens of 10 broilers, providing 16 replicates per treatment. Body weight and the amount of feed consumed was measured per pen on d 0, 21 and 42. Mortality was recorded daily. On d 43, one bird from each pen was sacrificed and breast and thigh samples were taken for n-3 FA analysis. No differences were observed between each group in terms of average weight gain, average feed intake or feed conversion ratio over the course of the study (d 0–42). Each increase in AURA inclusion corresponded to a significantly higher DHA content for both the thigh and breast tissue (thigh: 6.08, 16.19, 23.60 and 42.06 mg /100 g; breast: 4.26, 13.43, 22.68 and 36.30 mg DHA /100 g tissue for control, 0.25, 0.5, and 1% AURA, respectively, $P < 0.001$). The total n-3 FA content of breast meat was significantly higher ($P < 0.001$) in 0.5 and 1% algae compared with the control while no differences were observed between the groups in terms of the total n-3 FA content of thigh muscle. In contrast, no differences were observed between the groups in terms of the breast n-6 FA content, while 0.5 and 1% algae treatments significantly lowered total n-6 concentrations in thigh muscle ($P = 0.025$). In both thigh and breast tissue the n-6/n-3 ratio was reduced with each increase in AURA inclusion level (thigh: 12.34, 10.02, 8.28 and 6.28; breast: 11.94, 8.78, 7.14 and 5.50 for control, 0.25, 0.5, and 1% AURA, respectively, $P < 0.001$). Supplementation with AURA for 21 d improved the nutritional value of broiler breast and thigh tissues by increasing the DHA content and reducing the n-6/n-3 ratio in proportion to the level of AURA provided.

Key Words: algae, fatty acids, DHA, broiler

466P Anticoccidial efficacy of a live yeast (*Saccharomyces cerevisiae* CNCM I-1079) product fed to commercial broiler chickens exposed to a mixed challenge of *Eimeria species*. B. Lumpkins*[1], G. Mathis[1], V. Demey[2], and E. Chevaux[2], *[1]Southern Poultry Research Inc., Athens, GA, [2]Lallemand, Blagnac, France.*

Coccidiosis is a major enteric disease in the poultry industry that can lead to serious live performance issues as well resulting in necrotic enteritis. Anticoccidial drugs remain the most affordable and effective solution, but due to the pressure of the "all natural" market vaccination programs have increased and drug alternatives such as nutritional solutions must be evaluated. The potential of a live yeast (*Saccharomyces cerevisiae* CNCM I-1079, LY) product was evaluated using a randomized block design with 9 replications of 10 birds per cage. The treatments were: T1- nonmedicated non-infected (NMNI), T2- nonmedicated infected (NMI), T3- LY dose 1 (50g/mt), T4-LY dose 2 (150 g/mt), and T5- LY dose 3 (450g/mt). At day of hatch, male chicks (Ross 708) were allocated to cages and were feed treatment *ad libitum* throughout the experiment (D0–28). On D14, birds were challenged with *E acervulina* (EA), *E. maxima* (EM) and *E. tenella* (ET) at an average total of 150,000 oocysts except the NMNI birds. On D5 and D6 post-infection (PI), fecal OPG was counted and lesion scoring on D6 PI. The feed conversion PI (D14–20 and D14–28) was significantly improved ($P < 0.05$) for birds fed the diets with LY at doses 2 and 3 being the most consistent over time against NMI birds. In addition, the birds fed the LY at any of the dose levels had improved BWG compared with the NMI birds. Beyond improvements observed in live performance, the lesion scores of the birds fed any of the 3 LY doses were significantly reduced versus NMI birds. The greatest reduction in lesion scores was observed when birds were infected with either EM or ET when fed diets containing LY, and ET with the LY at dose 2 and 3 had the greatest reduction in Lesions. A negative correlation was reported for OPG with all LY doses, and EM being more sensitive at doses 2 and 3. Coccidiosis related mortality was numerically but non-significantly reduced by with the feeding of the yeast product at any of the dose levels. Specific nutritional solution such as the use of yeast products can help birds to mitigate coccidiosis negative effect, but the animal response appears to be dose dependent.

Key Words: live yeast, coccidiosis, *Eimeria*

467P Effects of dietary bee venom on immune response in broiler chickens challenged with or without lipopolysaccharide. D. H. Kim*[3], M. C. Keum[3], S. Lee[3], S. M. Han[1], H. Lillehoj[2], and K. W. Lee[3], *[1]National Academy of Agricultural Sciences, Wanju, Korea, [2]USDA, Beltsville, MD [3]Konkuk University, Seoul, Korea.*

This study was conducted to investigate the effect of purified bee venom on immune response in broiler chickens challenged with lipopolysaccharide (LPS). A total of 875 one-day-old feather-sexed male broiler chicks were randomly allocated to 5 treatments. A corn-soybean meal-based control diet was added with 0, 10, 50, 100, and 500 μg bee venom per kg of diet. Each treatment consisted of 7 replicates (25 birds/replicate) and reared for 28 d. Two birds per pen were selected for LPS injection. Each bird was subcutaneously injected with either 1 mg LPS per kg body weight or phosphate-buffered saline (PBS) at 28 d of age. At 4 h post LPS or PBS challenge, blood and spleen were sampled. Increasing dietary bee venom levels linearly or quadratically decreased ($P < 0.05$) serum concentrations of albumin, calcium, urea nitrogen, or creatinine. LPS challenge had higher ($P < 0.05$) levels of total cholesterol, triglyceride, glucose, albumin, total protein, calcium, phosphorus, creatinine, α-amylase, lactate dehydrogenase, uric acid, gamma(γ)-glutamyl transferase, and alkaline phosphatase in serum samples compared with the naïve control chickens. A significant interaction between dietary bee venom and LPS challenge on albumin levels was noted. Tumor necrosis factor α (TNFα) and α-1-acid glycoprotein levels in serum samples were

elevated ($P < 0.05$) in LPS-challenged vs naïve chickens. The expression levels of cytokines encoding for interleukin (IL)2, IL1β, IL6, IL8, IL15, IL18, TNFα in spleen were increased (linear or quadratic, $P < 0.05$) as dietary bee venom increased. LPS-challenge significantly lowered ($P < 0.001$) IL18, IL1β, IL6, IL8, IL18 transcript levels and improved ($P < 0.001$) IL2 and IL15 transcript levels in splenocytes. A significant interaction between bee venom and LPS with respect to the TNFα levels in spleen was noted. In conclusion, bee venom supplementation may alleviate the compromised immune status of broilers under immune stress induced by LPS challenge.

Key Words: bee venom, lipopolysaccharide, cytokine, serum parameters, broilers

468P The carryover effect of low dietary P and varying levels of myo-inositol on the metabolisable energy and nutrient retention in broilers. V. Pirgozliev* and S. Rose, *Harper Adams University, Newport, United Kingdom.*

An experiment was conducted with broiler chickens, which were previously fed on diets with and without the required available P content, and various levels of myo-inositol (MYO), to study the effects of previous exposure to dietary P and MYO on the N-corrected apparent metabolisable energy (AMEn), dry matter (DMR), and nitrogen (NR) retention coefficients of a standard diet fed to the birds at a later stage. The mucin secretions, measured as sialic acid (SA), and growth performance variables were also determined. Prior the experiment male Ross 308 broilers were reared in 60 floor pens (2 birds in a pen) from 7 to 21d age and fed one of 6 pre-experimental maize-soy-based mash diets. Two basal diets were formulated to be nutritionally adequate for chicks at that age (12.90 MJ/kg ME, 216 g/kg CP), as one of them was designed to have 4.8 g/kg non-phytate P, and the other diet had 2.5 g/kg non-phytate P. The 2 basal diets were then split in 3 batches each, and each batch was supplemented with MYO at 0.0, 3.0 and 30 g/kg diet, respectively to give a total of 6 experimental diets. Each diet was fed to 8 pens following randomization. At the start of the experiment, at 21d old all birds received different diet for 7 d, from 21d to 28d of age. The experimental diet was formulated to contain 12.56 MJ/kg ME, 200 g/kg CP, 9.4 g/kg Ca and 5.3 g/kg available P. Excreta were collected quantitatively for the last 4 d of the experiment and the studied variables were determined. A randomized block ANOVA was performed and a 2 × 3 factorial structure was used to investigate the main treatment factors (P × MYO levels) and their interaction. The values of the determined variables were in the expected range, and dietary AMEn, DMR, NR, and growth performance, did not differ ($P > 0.05$) in birds previously fed different P and MYO dietary levels. However, birds previously fed low P diet digested better ($P < 0.05$) the P and the Ca of the experimental diet. It has been demonstrated that a reduced intake of dietary P could increase the activity of intestinal alkaline phosphatase and phytase, thus supporting the observed results. The inclusion of MYO did not change ($P > 0.05$) the retention of Ca and P. There were no MYO × P interactions ($P > 0.05$) for any of the studied variables. In conclusion, the results suggest that a carryover effect of feeding low P diets in mineral retention can be expected although feeding MYO did not produce similar effects.

Key Words: myo-inositol, dietary P, ME, digestibility, previous exposure

469P Effects of increasing levels of guanidinoacetic acid diet supplementation on broiler performance during hot climate. E. Butolo*[1], J. Butolo[1], R. Sales[2], and L. Costa[2], *[1]Biojeb Instituto de Biociência, Mogi Mirim, SP, Brazil, [2]Granja Regina, Fortaleza, CE, Brazil.*

The purpose of this experiment was to determine the effect of guanidinoacetic acid (GAA) addition in a comercial broiler diets at increasing levels. GAA is synthesized in the liver and kidney from arginine (Arg) and glycine (Gly) and subsequently methyllated by S-adenosylmethione to creatine (Cr). Heat-stressed birds respond to the presence of higher uric acid concentration and changes in eletrolyte balance by increasing the usage of osmoprotectants. These osmolytes are viable endogenous precursors foy Gly. On the other hand, high supplemental lysine level on modern genetic broiler line could may alter the metabolic balance between amino acids, especially the arg:lys ratio. The amino acid arginine, as well as constituents of the protein, is involved in the synthesis of creatine and polyamines, proline, as a substrate for synthesis of collagen and nitric oxide (NO) and secretion of growth hormones (GH) and insulin-like growth factors (IGF). Three thousand day-old Cobb broiler chick were used for 41d floor pen trial following a complety randomized design of 6 levels of GAA (0; 0.05%; 0.1%; 0.15%; 0.2% and 0.25%) with 10 replicates of 50 (25 males and 25 females) birds each. The corn, soybean meal, meat and bone meal and fish meal was fed from d 0 to 41 d as Pre-Starter (250g/bird), Starter (650g/bird), Grower 1 (1100g/bird), Grower 2 (1400g/bird) and finisher (1000g/bird) during the rain season at the Northeast of Brazil (temperature above 30°C with high humidity). The results of body weight (BW), feed conversion (FC) and feed intake (FI) of birds fed increased levels of GAA were, repectively, 2487, 2498, 2500,2507,2508 and 2513 g of BW; 1.668, 1.66, 1.653, 1.651,1.645 and 1.643 of kg/kg of FC; 4192, 4188, 4168, 4170, 4172 and 4174 g/bird of FI. Dietary treatments showed linearly increase in weight gain ($Y = 2.4856+0.00482X$; $R^2 = 0.9535$) and linear reduction on feed conversion ($Y = 1.6713–0,00495X$; $R^2 = 0.9784$). The feed intake was not affect for treatments ($P > 0.05$). The inclusion of increasing levels of guanidinoacetic acid, up to 0.25% in the diet, linearly increases the performance of broiler chickens up to 41 experimental days.There is a possibilty that broilers raised under the hot and humid climate require higher Gly and Arg fortification and the addition of guanidinoacetic acid can save arginine and glycine and may yield performance responses.

Key Words: guanidinoacetc acid, broiler, performance, nutrition, diet

470P Performance and carcass yield of broilers fed diets formulated by prediction equations of metabolizable energy of animal's meals. C. Oliveira[1], C. B. V. Rabello*[1], E. Lopes[1], B. Silva[1], E. Soares[1], M. José dos Santos[1], D. Oliveira[1], D. da Silva[1], and P. Rodrigues[2], *[1]Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brazil, [2]Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil.*

A practical and economical way of estimating energy values is the use of prediction equations, established per chemical composition of the food, these equations can also be obtained through meta-analysis of the literature. The objective of this study was to validate prediction equations for corrected apparent metabolizable energy (AMEn) of animal's meals. The performance experiment was carried out in a total of 648 broilers distributed in a completely randomized design in 2 × 3 factorial scheme (2 animal's meals, poultry offal meal (POM) and meat and bone meal (MBM), and 3 methods of AMEn determination of these meals A, B and C and 6 replications of the 18 repetitions. AMEn were estimated by chemical composition of the feeds: crude protein (CP), ether extract (EE), mineral matter (MM), crude energy (CE), calcium (Ca) phosphorus (P) and Age. The method A- Prediction equations obtained in metabolism assay EMAn = 6802 - 213.3 EE - 127.3 Ca + 10.47 Age for POM and EMAn = 2789–72 EE + 14 Ca + 20.28 Age for MBM; Method B- Prediction equations obtained with meta-analysis of the chemical composition by literature to POM AMEn = 6139 - 45.5 CP + 0.356 CB – 123.5 MM and for MBM AMEn = 2267 + 19.9 CP + 67.9 EE - 44.4 MM; and method C- Diets elaborated with AMEn values by Brazilian tables of feed composition. The variables of performance and carcass yield were evaluated by ANOVA and Tukey test ($P < 0.05$). There was no interaction between the factors studied in any

ages, but in the 1 to 7 d old, feed intake (FI) was significant for meal factor, being highest for the diets containing POM (150,31 g/bird), and feed conversion (FC) was best for diets with MBM (1,033 g/g), as well in the 1 to 21 d old (1,411 g/g). This result may be related to the different levels of fat and amino acid profile (non-essential amino acids) used in the diets, resulting in better FC. At 1 to 35 and 1 to 42 d old, there was no significant difference for the performance variables. On the other hands, breast yield was significantly highest for the broilers fed with POM diets, however, thigh and drumstick yields were highest for broilers fed with diets containing MBM . The variation of the body tissues yield may occur by amino acid digestibility and profile in the diets, which may influence the amount of amino acids available used in the process of protein synthesis. Yield or absolute organ weight and abdominal fat wasnt influenced by treatments. The results suggest that prediction equations tested can be used for predict AMEn values of the POM and MBM in broilers diets.

Key Words: energy value, meat and bone meal, poultry offal meal, metabolizable energy, broiler

471P GC-FID method validation and stability assessment of DHA in chicken tissues. G. Dillon[2], W. Brandl[1], C. Cardinall[1], W. Yuan[1], T. Ao*[3], A. Yiannikouris[3], and C. Moran[2], *[1]Mérieux Nutrisciences, Burnaby, BC, Canada, [2]Alltech SARL, Vire, France, [3]Alltech Inc., Nicholasville, KY.*

A GC-FID method was validated and verified for its fitness in analyzing 7 fatty acids in 4 different chicken tissues: breast, thigh, skin, kidney and liver. The characteristics studied for validation included linearity, accuracy, LOD and LOQ, specificity, and stability. Commercial reference standards (NIST SRM 3275–3 omega33 concentrate and NIST 1546a meat homogenate) were also analyzed to demonstrate the suitability of the method. Sample preparation involved grinding the tissue followed by freezing and freeze-drying for 48 h. Chicken skin was prepared by placing in liquid nitrogen before freeze-drying. Freeze-dried samples were extracted and methylated to produce the methyl ester of the fatty acids (FAME), which were then analyzed by GC-FID. The method was validated over a linear range of 0.3 mg/ml to 30 mg/ml with coefficients of determination ranging between 0.997 and 1.000. Accuracy experiments were conducted on all 5 chicken tissues. The endogenous levels of $C_{16:0}$, $C_{18:1C}$ and $C_{18:2C}$ were too high to calculate recovery on the spikes however, the recoveries for $C_{18:1T,}$ $C_{18:2T}$, $C_{20:5}$ and $C_{22:6}$ were within the acceptable limits of ± 20%. Reproducibility for each analysis was within the acceptance criterion of < 5%. Specificity of the method was addressed for each targeted analyte by using their corresponding FAME analytical standards and by assessing low-level spikes in the different matrices to evaluate potential interferences and distinguish analytes from other substances. The robustness of the method was established by comparing data obtained from 2 different analysts demonstrating that validation parameters were all within acceptable limits. The stability of test solutions and samples were carried out establishing that the FAME stock solutions were stable after 6 mo at −16°C. The short-term stability of the extracts was confirmed for all matrices after 2, 24 and 48 h, at 20°C, while the long-term stability of freeze-dried tissues of breast, thigh and skin stored at −16°C was confirmed up to 16 wks. Kidney and liver were found to be stable for 4 and 8 wks, respectively. The freeze and thaw stability of each matrix was confirmed over 4 cycles. In conclusion, the method has been deemed fit for the purpose of measuring concentrations of $C_{18:1T,}$ $C_{18:2T}$, $C_{20:5}$ and $C_{22:6}$ in chicken tissues for regulatory studies. The stability of analytes in tissues has been also established

Key Words: DHA, chicken, tissue, validation, stability

472P Comparative nutritive value of a co-extruded mixture of flaxseed and pulses (linPRO) fed with or without multi-carbohydrase to broiler breeder hens and broiler chicks. A. Thanabalan*[1], J. Moats[2], and E. Kiarie[1], *[1]University of Guelph, Guelph, ON, Canada, [2]O&T Farms Ltd., Saskatoon, SK, Canada.*

Nutritive value of feed ingredients are typically determined using broiler chicks assay. However, it is not well known whether differences in digestive tract maturity and feeding schedule may influence nutritive value of feed ingredients. This study evaluated standardized ileal digestibility (SID) of AA and AMEn in linPRO (extruded full-fat flaxseed and ground pulses) fed to broiler breeder hens (BB, Exp. 1) and broiler chicks (BC, Exp. 2) with or without a multi-carbohydrase (MC). The MC supplied 1,200 U of xylanase, 600 U of β-glucanase, 2,800 U of cellulase, 400 U of mannanase, and 2,500 U of amylase per kg of feed. Diets contained 0.5% TiO_2 as an indigestible marker and were fed in mash form. In Exp. 1, diets were a corn soybean meal basal diet formulated to meet breeder specifications and basal diet with energy- and AA- yielding ingredients replaced with 18% linPRO with or without MC. Sixty, 26 week old Cobb 500 BB were placed in cages (2 birds/cage), allocated to diets (n = 10) and fed once daily according to breeder feeding curve for 30 d. Excreta samples were collected from d 28–30 and all birds were sacrificed on d 30 for ileal digesta. In Exp. 2, a semi-purified cornstarch diet containing 90% linPRO was prepared with or without MC. N-free diet was also fed to estimate basal endogenous AA losses (AAL). A total of 240 d old Ross 708 male chicks were placed in cages and fed commercial starter diets until d 13. On d 13, chicks were weighed, allocated to 24 cages (10 birds/cage), and fed experimental diets to d 21 (n = 8). Excreta samples were collected from d 18–21 and all birds were sacrificed on d 21 for ileal digesta. The SID of AA and AMEn in linPRO in Exp. 1 were determined using the difference method and the corresponding values in Exp. 2 were determined using direct and difference methods, respectively. The basal AAL determined in Exp. 2 were applied in both experiments. There was no interaction between age and MC or main effect of MC on SID of AA, however, the main effect of age was such that SID of AA in linPRO were higher ($P < 0.01$) in BB. Among indispensable AA, Thr (88.6%) was least digestible and Phe (97.3%) was most digestible in BB whereas Ile (63.1%) was the least digestible and His (76.5%) was most digestible in BC. There was no interaction between age and MC or MC on AMEn; the main effect of age was such that BB had higher ($P < 0.01$) AMEn than BC. The AMEn was 4,012 and 2,105 kcal/kg for BB and BC, respectively. In conclusion, BB had higher SID of AA and AMEn than BC, suggesting differences in digestive tract capacity and the impact of feeding schedule on nutrient utilization in linPRO. Supplemental MC did not influence utilization of nutrients in linPRO.

Key Words: linPRO, broiler breeder, broiler chick, SID of AA, AMEn

473P Prediction equations for energy values of animal-derived meals obtained by meta-analysis. C. Oliveira[1], C. B. V. Rabello*[1], E. Lopes[1], R. S. Junior[1], G. Macambira[1], M. José dos Santos[1], D. Oliveira[1], D. da Silva[1], and P. Rodrigues[2], *[1]Universidade Federal Rural de Pernambuco, Recife, Brazil, [2]Universidade Federal de Lavras, Lavras, Brazil.*

In the search for more consistent prediction equations about the energy and nutritional values of ingredients such as animal meal, we can use the meta-analysis principle, which is based on the synthesis of data from different published with related studies in the formulation of a statistical model that better explains the observations, generating new results. The objective of this study was to determine prediction equations to estimate the corrected apparent metabolizable energy (AMEn) values of poultry

offal meal (POM) and meat bone meal (MBM) used in broiler diets through meta-analysis. A bibliographic review was undertaken with studies conducted in Brazil from 2000 to 2016 to catalog information on AMEn values and the following chemical elements in the composition of the feedstuffs: crude protein (CP), ether extract (EE), gross energy (GE), mineral matter (MM), calcium (Ca) and phosphorus (P). The studies included in the database amounted to 6,299 broilers, whose average initial age was 16 d old (ranging from 1 to 41 d old) and average final age was 25 d old (ranging from 7 to 50 d). Most part of the studies (57%) used male broilers, 7% used females, and 36% involved mixed batches. The total excreta collection methodology was adopted in 86% of the researches, and the forced-feeding method with roosters was used in 14% of them. The level of 20% inclusion of the test feedstuff in control diet was indicated by 40% of the papers. Groups were also cataloged and formed according to bird sex and age. The chemical correlations were analyzed and a multiple linear regression model with the Stepwise procedure to examine the association between the variables, which were included in the equation as a function of their importance. The high and significant correlation coefficients between the independent variables GE, MM, CP, EE, Ca and P and the dependent variable AMEn contribute to the understanding of variations in the energy values of these feedstuffs. According to the coefficients of determination, the best equations to estimate the AMEn of POM and MBM were: AMEn = 6139 − 45.5 CP + 0.356 GE − 123.5 MM (R^2 = 0.83) and AMEn = 2267 + 19.9 CP + 67.9 EE − 44.4 MM (R^2 = 0.90), respectively. On the basis of these results, the correlations among chemical components, the variability of animal-derived feedstuffs, and the criterion adopted in the choice of mathematical models to determine the energy values of animal-derived meals are critical factors for a successful of diet formulation.

Key Words: broiler, meat and bone meal, metabolizable energy, poultry offal meal, prediction equation

474P The addition of selected full-fat insect meals affects the gastrointestinal tract microbiota in broilers. B. Kieronczyk*[1], D. Józefiak[1], A. Józefiak[1], M. Rawski[1], A. Benzertiha[1], S. Talibov[1], S. Nogales-Merida[1], P. Gobbi[2], and J. Mazurkiewicz[1], *[1]Poznan University of Life Sciences, Poznan, Poland, [2]HiProMine S.A., Robakowo, Poland.*

Insects are considered as a potential protein source for poultry and companion animals. The crude protein (CP) content of insects is species-dependent and varies from 40 to 60% in dry matter (DM) content. Several studies were conducted with selected insect species used in animal production, i.e., *Tenebrio molitor* (mealworm), *Hermetia illucens* (black soldier fly) and *Musca domestica* (housefly), as a source of protein. The aim of the study was to evaluate the effect of selected full-fat insect meals fed "on top" to broiler chickens on the gastrointestinal tract microbiota. In the trial, 400 one-day-old, female ROSS 308 chicks were used. Birds were placed into 4 treatments containing 10 replicates per group and 10 birds per pen. *S. lateralis* (SL20), *T. molitor* (Linnaeus, 1758) (TM20) and *H. illucens* (Linnaeus, 1758) (HI20) were used at 0.2%. Populations of microbiota were determined in the crop, ileum and cecal digesta by fluorescent in situ hybridization. All details of sample preparation and FISH analyses for bacteria enumeration from digesta are described in Józefiak et al. (2013). Data were tested using the GLM procedure of the SAS statistical software package and means were separated using Duncans' tests following one-way ANOVA. Irrespective of the GIT segment, digesta pH was not altered by the application of different full-fat insect meals; however, even in the crop, statistically significant changes in microbiota were observed. In the TM20 treatment, Bacteroides–Prevotella cluster were observed (P = 0.001). Compared with SL20 and TM20 treatments, HI20 decreased *Clostridium leptum* subgroup counts and increased those of the *Clostridium coccoides–Eubacterium rectale* cluster. Moreover, in the HI20 treatment, *Lactobacillus* spp./*Enterococcus* spp. increased compared with those of the negative control (NC) and SL20 treatments. In the ileal digesta, *C. coccoides–E. rectale* cluster count was hight among all treatments, but the results were only significantly different compared with the NC results (P < 0.001). HI20 treatment decreased *Lactobacillus* spp./*Enterococcus* spp. counts, whereas an increase was noted in TM20 treatment. In the cecal digesta, counts of Bacteroides–Prevotella, *C. coccoides–E. rectale* clusters. HI20 addition also resulted in increased counts of *Lactobacillus* spp./*Enterococcus* spp. compared with those in the control and TM20 group. Based on the results of the experiment, we conclude that supplementation with full-fat insect meals in relatively small amounts, i.e., 0.2%, in the diet of broiler chicken can have a positive effect on microbial composition in of the GIT.

Key Words: insect meal, feed additive, broiler chicken, GIT microbiota

475P The estimation of body composition of broilers chicken from 7 to 35 days of age by dual energy x-ray absorptiometry (DXA). M. Hamdi*, I. Lachance, and M. P. Létourneau-Montminy, *Université Laval, Ville de Québec, QC, Canada.*

The ability to accurately measure body or carcass composition is important for performance testing, requirement determination and carcass yield. The use of non-invasive procedures like dual-energy x-ray absorptiometry (DXA) is interesting over invasive procedure like dissection and chemical analysis. The objectives of the current study were to 1) validate the use of DXA (Discovery W; Hologic Inc., Waltham, MA, USA) in evaluating the body composition in fat FatDXA), lean (LeanDXA), and bone mineral content (BMC), of broilers receiving different levels of dietary Ca, P, crude protein and energy, and 2) to develop prediction equations for each of these parameters according to the results obtained from the DXA scan. A total of 135 one-day-old male broilers (Cobb500) were distributed into 3 experimental treatments (3 pens/treatment, 45 birds/pen) and body weight was recorded weekly. Experimental treatments were designed to induce body composition variations which is necessary to have a robust equation. They consisted in a control diet that fulfilled all nutrient requirements with antibiotic growth factors, a diet without meat and bone meal and antibiotics, and a diet reduced in Ca, P and metabolizable energy. On d 7, 14, 21, 28 and 35 post-hatch, 3 birds per replicate were euthanized, frozen and then scanned in group of 3 using DXA with the rat whole body mode for the chicken at 7 and 14 d and the infant whole body for the 21, 28 and 35 animals. Broilers carcass were frozen and homogenized by grinding before chemical determination of lipid, protein, calcium (Ca) and phosphorus (P). Dry body lipids and protein ranged from 6 to 16% and from 12 to 20%, respectively. Total carcass fat (FatC), protein (ProtC), Ca (CaC) and P (PC) were predicted by regression analysis using measurements from DXA as an independent variable. Chemical data were all predicted with good accuracy: ProtC (g) = −10.4 + 0.21 LeanDXA (g), R^2 = 0,97; FatC (g) = 11.4 + 0.49 FatDXA (g), R^2 = 0.95; CaC (g) = −0.218 + 0.38 BMC (g), R^2 = 0.96; PC (g) = −0.086 + 0.29 BMC(g), R^2 = 0,97). These results indicate that DXA is a reliable method to predict body composition which has tremendous potential for the poultry industry with results that can be rapidly analyzed. It's also a valuable tool for monitoring bone mineralization.

Key Words: broiler, DXA, calcium, phosphorus, lean

476P Nutrient characteristics of corn distillers dried grains with solubles. A. Pekel*[1], M. Alatas[2], O. Sayin[3], E. Kuter[3], and O. Cengiz[3], *[1]Faculty of Veterinary Medicine, Istanbul University, Istanbul, Avcılar, Turkey, [2]Faculty of Veterinary Medicine, Selcuk University, Konya, Turkey, [3]Faculty of Veterinary Medicine, Adnan Menderes University, Aydin, Turkey.*

This study was conducted to determine the nutrient composition and color characteristics (L,* a,* b*) of corn distillers dried grains with soluble (DDGS) collected from different feed mills in Turkey (n = 18). Dry matter, ash, ether extract, crude protein (CP), crude fiber, neutral detergent fiber (NDF), and acid detergent fiber (ADF) content of the samples were determined. Color (L,* a,* b*) was evaluated using a Minolta Chroma Meter. Proc corr and reg procedures were used to determine significant correlations between parameters. Average crude ash, ether extract, and crude protein were 5.37%, 10.88%, and 30.54% on a DM basis, respectively. Average crude fiber, ADF and NDF content were 10.08%, 22.67%, and 42.49% on a DM basis, respectively. Among the nutrients, ether extract, crude fiber, and ADF had the highest variation with having coefficient of variation (CV) values of 23, 12, and 12, respectively. There were significant correlations between ADF and L* ($r^2 = 0.46$), between CP and b* ($r^2 = 0.41$), and between CP and ether extract ($r^2 = 0.40$), respectively. By regressing the CP content against the ether extract content and b* level, the regression equation was CP = 38.75522−0.192 (Ether extract)−0.187(b*), $r^2 = 0.58$, which suggested a significant ($P < 0.01$) relationship to estimate CP content from ether extract and b* level of corn DDGS. Multiple linear regression of ether extract on CP and a* level resulted in the following equation ($P < 0.01$): Ether extract = 61.504−1.14 (a*)−1.25(CP), $r^2 = 0.56$. In conclusion, there was considerable variation in ether extract and fiber content and the significant multiple regression equations can be used to estimate CP and ether extract content of corn DDGS.

Key Words: color characteristic, corn distillers dried grains with solubles, correlation, nutrient composition

477P Determining the effects of feeding two broiler strains varied crumble size and intact pellets (d 0-18) on d 63 processing characteristics. M. Lemons*[1], C. McDaniel[1], J. Moritz[2], and K. Wamsley[1], *[1]Mississippi State University, Mississippi State, MS, [2]West Virginia University, Morgantown, WV.*

Our laboratory found that feeding an average crumble (C) particle size of 2200 to 3736 µm improved starter broiler performance; with the greatest benefit being associated with the largest particle size tested (3736 µm). However, these trials only examined starter performance without looking at the effects on overall performance and processing characteristics. This led to a companion study examining the effects of varying C particle sizes and different intact pellet (IP) percentages during the starter growth phase (d 0–18) on starter and overall (d 0–62) performance using 2 commonly used genetic strains [S; (fast growing-SA or high yielding-SB)]. Therefore, the current study was performed to evaluate the effects of feed form (FF) and feed quality (FQ) fed in the starter phase when fed to 2 S (SA or SB) on d 63 processing characteristics. The current study employed a 2 (S) × 2 (FF) × 3 (FQ) factorial arrangement within a RCBD. Crumbles or pellets (FF) were fed as one of 3 different FQ, which varied dependent on FF. These FQ included: Low-2210 µm (C) or 40% IP, Medium-3010 µm (C) or 60% IP, and High-3388 µm (C) or 80% IP. One nutritionally common diet was manufactured at a commercial mill with modifications performed to adjust desired FQ for each FF. Common diets (ranging from 65 to 75% IP dependent on growth phase), also manufactured at a commercial mill, were fed in subsequent growth phases (>18 d). Two broilers of each sex per pen were randomly chosen for determination of d 63 processing characteristics. Tissue weight, yield relative to body weight (YBW), yield relative to carcass weight (YCW), and wooden breast severity were determined. Significant S × FF × FQ interactions were present for pectoralis major YCW and YBW ($P < 0.05$). For SA, broilers provided 40% IP resulted in the highest YCW with broilers receiving 3010 µm C having the lowest YCW; other treatment combinations performed intermediate. However, SB had an improved YCW when providing 2210 µm crumbles or 80% IP. A similar response was apparent for pectoralis major YBW, with the exception of SA demonstrating a lower YBW for "high" FQ of both FF. Interactions for wooden breast severity were not present, but the main effect of FQ demonstrated a higher incidence when "Low" FQ was presented ($P = 0.006$). These results demonstrate that S × FF × FQ interact to influence processing characteristics suggesting integrators should provide 40 or 80% IP in the starter phase (d 0–18) for improved pectoralis major yields, dependent upon the S they are utilizing. However, integrators using SA should be cognizant of providing 40% IP from d 0–18 to improve pectoralis major yields due to the presence of "low" FQ increasing wooden breast severity.

Key Words: processing, pellet, crumble, particle size, feed form

478P The effect of fermented rapeseed cake on the growth performance of broiler chicken. D. Józefiak*[1], A. Zaworska[1], M. Potocka[1], B. Kieronczyk[1], M. Rawski[1], A. Benzertiha[1], M. Pachocka[2], and J. Sypniewski[2], *[1]Poznan University of Life Sciences, Poznan, Poland, [2]PIAST PASZE Sp.z o.o, Ostrów Wlkp., Poland.*

In Poland and in Europe, the deficit of feed protein sources has been a problem present for years. This became clear in the adoption of the European Parliament's own-initiative resolution 'The EU's protein deficit' on 8 March 2011. But the inclusion levels of rapeseed materials to poultry diets are limited due to the significant participation of the crude fiber and antinutritional factors – (glucosinolates, myrosinase, p-phytic) and their relatively low energy value. The aim of this study was to determine the effect of the use of rapeseed feed on broiler chickens performance. The experimental material comprised 216 1-d-old broiler chickens Ross 308. The birds were randomly allocated to 3 dietary treatments (12 replicates, 72 birds per replicate). The experiment lasted 6 weeks. Diets for each period were formulated to be isonitrogenous and isocaloric. The diets were prepared in mash form. In the control group (C), the main source of dietary protein was soybean meal (SBM). In experimental diets, SBM was partially replaced with 15% of raw (T1) or fermented rapeseed cake (T2). During the experiment body weight control (BWG), feed intake (FI) was recorded and feed conversion ratio (FCR) calculated Data were tested using the General Linear Models (GLM) procedure of SAS software.In the experiment, means were separated using Duncans' tests following one-way ANOVA. There were no statistically significant differences in BWG and FI over a 7–35 d period ($P = 0.307$, $P = 0.462$ respectively), but the growth animals fed with the T2 group was 6% better than in T1 group and 5% better than in C group. It was found that the animals from the T2 group significantly ($P = 0.05$) better use of feed than the C group, and 4% better than with T1 group. To conclude, the research conducted to date has shown that fermented rapeseed feeds can serve as a replacement of soybean meal in broiler chickens.

Key Words: rapeseed feed, fermentation, broiler chicken, growth performance

479P Energetic values of corn germ meal for broilers evaluated by the response surface model. E. Lopes, C. B. V. Rabello, M. José dos Santos*, C. Oliveira, G. Macambira, D. da Silva, and D. Oliveira, *Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brazil.*

The corn germ meal (CGM) is from industrialization of corn by wet milling process. This by-product does not undergo oil extraction process, maintaining it's in high lipid (59.8 and 44.33% maximum and minimum, respectively) levels. The objective of this research was to evaluate the energy values, metabolizable coefficients and the relationship between age and level of CGM (55% of lipid) replacement at diets for broilers. 720 broilers (Cobb 500) one day old were distributed in 3 trials: pre-initial (1 to 8 d old, 10 birds by replicates), initial (14 to 21 d old, 6 birds by replicates) and growth I (25 to 33 d old, 4 birds by replicates).The broilers were allocated in cages in a completely randomized design with 6 treatments and 6 replicates.The treatments were: reference diet (corn and soybean meal) and 4 diets with 10, 15, 20, 25 and 30% of replacement of the reference diet by CGM. The methodology of partial excreta collection were used to determine the apparent metabolizable energy (AME) and for nitrogen balance (AMEn) and the apparent metabolizable coefficients of crude energy (AMCCE), dry matter (AMCDM) and ether extract (AMCEE) of CGM. The data was fit for response surface model, considering the age of the broilers and CGM levels as independent variables. The stationary point for AME and AMEn was 4173 kcal/kg (AME = 1781.1668 + 52.4459age + 203.4546 level - 1.5704 level × age −4.1196 level^2, R^2 = 0.72), 3591kcal/kg (AMEn = 1551.2551+ 68.4218 age + 196.6864 level – 1.8188 level × age −3.8494 level^2, R^2 = 0.76).To AMCCE, AMCDM, AMCEE the stationary point was 49.3, 72.6 and 61.3%, respectively. The models were: AMCCE = 22.0186 + 0.8959 age + 2.5993 level – 0.0239 level × age - 0.0511 level^2 (R^2 = 0.76), AMCDM = 2.1622 age + 5.4712 level– 0.0213 age^2– 0.0505 level × age - 0.1177 level^2 (R^2 = 0.43) and AMCEE = 27.3678 + 1.8211 age + 5.3127 level – 0.0202 age^2 - 0.1181 level^2 (R^2 = 0.68). The stationary point for AMEn was at 18 d old with 28% of CGM. There was a correlation between age × level for all analyzed variables, except for AMCEE. The dependence between the factors indicates that the energy utilization of the GIM by broilers varies according to the replacement level and the age of the broilers.

Key Words: by-product, digestibility, lipid source, metabolizable energy

480P Egg quality of laying hens fed different levels of shiitake residue (*Lentinula edodes*). R. F. Netto*, G. Pasquali, J. Batistioli, L. Zanetti, T. Santos, E. Muro, L. Dornelas, A. C. Neto, R. G. Giacomini, F. B. Júnior, D. Souza, J. Denadai, and J. R. Sartori, *São Paulo State University (UNESP), São Paulo, Brazil.*

Shiitake (*Lentinula edodes*) is the second most produced mushroom in the world with an estimated production of 5 thousand tons per year in Brazil. There are no studies concerning the inclusion of shiitake residues (stems and fruiting body) in animal nutrition. This residue has similar composition and bioactive compounds like shiitake marketed for human consumption. The mushrooms are source of prebiotics like non-starch polysaccharides with therapeutic activities, such as anticarcinogenic, anti-inflammatory and antibiotic. In addition, shiitake has a high nutritional value containing vitamins B1, B2, B12, C, D, folate, niacin and minerals (3.7 to 7.0%). The objective of this study was to evaluate the effects of different shiitake residue levels inclusion on egg quality of brown-egg laying hens. A total of 192 Lohmman Brown hens were housed in cages from 60 to 72 weeks old. A 16-h light program was used. The laying hens received water and food *ad libitum*. The experimental design was completely randomized, with 4 dietary treatments (control, 1.0, 1.5 and 2.0% of shiitake residue meal) with 6 replicate cages of 8 birds each. The parameters evaluated were: albumen height, yolk (height, weight and color), Haugh unit, especific gravity and shell thickness. Data were collected in 3 4-week periods and analyzed by GLM procedure of Minitab 17. According to the results, different levels of shiitake residue in laying hens diets did not affect the egg quality ($P > 0.05$). Our results suggest that shiitake residue can be included up to 2% in laying hens diets without impairment of performance.

Key Words: brown-egg layer, feed additive, egg, mushroom, shiitake by-product

481P Standardized ileal digestible amino acids and apparent metabolizable energy content in defatted black soldier fly larvae fed to broiler chickens. Z. Mwaniki* and E. Kiarie, *University of Guelph, Guelph, ON, Canada.*

Black soldier Fly (*Hermetia illucens*) products were recently approved as novel poultry feedstuff in Canada, US and European Union. However, research in practical poultry feeding is still in its infancy and manufacturers continue to innovate production processes to improve meal quality. Characterization of the nutritive value of emerging products will be critical for accurate feed formulation and successful incorporation in poultry feeding programs. We carried out an experiment to determine apparent ileal digestibility (AID), standardized (SID) ileal digestibility of AA and apparent metabolizable energy (AME) in defatted black soldier fly larvae meal (BSFLM) fed to broiler chicks. The sample of BSFLM (Enterra Feed Corp., BC, Canada) contained 59.3, 7.0 and 6.1% DM for CP, fat and starch, respectively. A total of 180 d old male broiler chicks (Ross 708) were placed in 12 cages and fed a commercial broiler starter diet to d 13 of age. On d 14, birds were weighed and placed in cages (10 birds/cage; n = 6) and allocated one of 2 semi-purified cornstarch-based diets. The diets were: a N free diet (NFD) for estimating endogenous AA losses and a 20% CP test diet with BSFLM as the sole source of N and AA. All diets had 0.5% TiO_2 as an indigestible marker and the ratio of cornstarch to sucrose and soy oil in test diet was identical to NFD to calculate AME by difference method. Birds were given feed and water *ad lib*. Excreta samples were collected on d 17–20. On d 21, all birds were sacrificed for ileal digesta. Data was presented in terms of Means and CVs. Analyzed indispensable AA concentrations in BSFLM sample were: 5.6, 3.9, 3.5, 3.3, 2.8, 2.4, 2.3, 2.2, 0.9%DM for His, Leu, Val, Lys. Arg, Ile, Thr, Phe and Met, respectively. The AID and SID of CP in BSFLM was 80.7 and 84.6%, respectively. Among the indispensable AA, Arg had the highest AID (88.7%) and SID (92.0%) values and His had the least AID (54.6%) and SID (61%) values. The SID of Lys, Met, Cys, Thr, Ile, Val were 86.3, 88.7, 72.8, 85.5 and 88.6%, respectively. The SID CP content was 47.5% DM with a CV of 0.6%. The SID content of His and Leu were highest (3.5%DM) and for Met (0.82% DM) and Cys (0.30% DM) lowest and had the highest CV, 3.6% for Met and 5.3% for Cys. Apparent retention of gross energy in BSFLM was 64.5% with a CV of 3.5%. AME was 3,288 kcal/kg DM with minimum and maximum of 3,127 and 3,422 kcal/kg DM, respectively. The data indicated BSFLM had low sulfur amino acids concentration with high variability in terms of utilization in broiler chickens. Since sulfur amino acids are the most limiting in poultry feed, consideration for fortification will be required while incorporating BSFLM in poultry feed programs.

Key Words: black soldier fly larvae meal, SID of AA, AMEn, broiler

482P Standardized ileal digestibility of amino acids and apparent metabolizable energy in processed soybean meal (Alpha-Soy) fed to broiler chicks. E. Kim*[1], Y. Rho[1], H. M. O'Neill[2], H. Schulze[2], and E. Kiarie[1], *[1]University of Guelph, Guelph, ON, Canada, [2]AB Agri Ltd., Peterborough, United Kingdom.*

Processed soybean meal with reduced anti-nutritional factors that are commonly used in piglet pre-starter diets may be helpful in bolstering post-hatch gut resiliency to luminal challenges in chicks. However, application of such products requires determination of digestible amino acids and energy for accurate feed formulation. We carried out an experiment to determine apparent (AID), standardized (SID) ileal digestibility of AA and apparent metabolizable energy (AME) in processed soybean meal (PSBM, AlphaSoy) fed to broiler chicks. The PSBM sample was tested alongside a sample of conventional soybean meal (SBM) that had been used as the starting material for production of PSBM using enzymatic pre-treatment. A total of 270 d old male broiler chicks (Ross 708) were placed in 12 cages and fed a commercial broiler starter diet to d 13 of age. On d 14, birds were weighed and placed in cages (10 birds/cage; n = 6) and allocated one of 3 semi-purified cornstarch-based diets. The diets were: a N free diet (NFD) for estimating endogenous AA losses and 2 20% CP test diets formulated with either PSBM or SBM as the sole source of N and AA. All diets had 0.5% TiO_2 as an indigestible marker and the ratio of cornstarch to sucrose and soy oil in test diet was identical to NFD to calculate AME by difference method. Birds were given feed and water *ad lib*. Excreta samples were collected on d 17–20. On d 21, all birds were sacrificed for ileal digesta. On DM basis, the GE was 4,481 and 4,290 in PSBM and SBM, respectively and corresponding values for CP were 49.6 and 48.9%, respectively. The AID of CP and AA except Arg and Cys was higher ($P < 0.05$) in PSBM compared with SBM. The SID of CP was higher in PSBM sample (91.6 vs. 89.6%, $P = 0.04$). Among the indispensable AA, chicks fed PSBM showed higher ($P < 0.05$) SID of His, Leu, Phe, and Val than SBM sample. Tendencies were observed for higher SID of Lys (94.1 vs. 92.5%, $P = 0.06$) and Ile (89.3 vs. 88.3%, $P = 0.052$) in PSBM sample compared with SBM sample. The largest differences between SID values of indispensable AA was observed for His (2.5 percentage units, $P = 0.002$). The mean SID value of indispensable AA was higher for PSBM (91.0 vs. 89.8%, $P = 0.024$) than in SBM. The SID of Ala, Asp, Glu, Gly, Pro and Tyr were higher in PSBM than in SBM sample. The apparent retention of gross energy was higher ($P = 0.004$) in PSBM (68.0 vs. 61.7%) than SBM. The AMEn for PSBM and SBM was 2,543 and 2,205 kcal/kg as received. The results indicated that processing soybean meal improved nutrient and energy utilization indicating improved nutritive value in broilers.

Key Words: processed soy bean meal, SID of AA, AME

483P Total tract non-cellulosic polysaccharide digestibility in broilers at d 21 with corn-soy based diet versus DDGS based diets. P. Maharjan*, J. Caldas, K. Hilton, M. Cortes, M. Schlumbohm, G. Mullenix, A. Beitia, J. Weil, J. England, and C. Coon, *University of Arkansas, Fayetteville, AR.*

A study was conducted to understand total-tract non-cellulosic polysaccharide (NCP) digestibility in d21 broilers fed diets based on corn-soy and corn DDGS based diets (DDGS 1 and DDGS 2 from 2 different suppliers). Diets were formulated to meet the nutrient requirements as suggested by Primary Breeder. Birds were reared in digestibility cages and pooled excreta samples (n = 5 birds per cage) were taken for replicate cages (n = 6). Test diets and pooled excreta samples for treatment diets were analyzed for NCP content as soluble NCP (S-NCP) and insoluble NCP (I-NCP) fractions in diets and excreta. Digestibility coefficient (DC) values were then determined for treatment diets and significantly different DC values were separated using HSD test (JMP Pro 13) at $P < 0.05$. Results showed the total-tract digestibility for corn soy based diet was 0.69 for I-NCP fraction and 0.83 for S-NCP fraction. The corn soy based diets produced higher DC values ($P < 0.05$) for both NCP fractions compared with the DDGS based diets. Total-tract DC values analyzed for 2 DDGS based diets were 0.58 and 0.56 for I-NCP and 0.61 and 0.56 for S-NCP fractions. At monosaccharide level from NCP, glucose had the highest DC values for S-NCP and I-NCP (0.95 and 0.77) for corn soy based diet, whereas glucose DC values were lower for DDGS based diets (0.78 and 0.71 for DDGS 1 and 0.43 and 0.63 for DDGS 2, for S-NCP and I-NCP respectively). Arabinoxylan DC value was higher for corn-soy based diet (0.57) compared with DDGS based diets (0.54 and 0.53 for DDGS 1 and DDGS 2). Overall, NCP digestibility for corn-soy based diet was found to be higher than DDGS based diets.

Key Words: non-cellulosic polysachharide, corn-soy based diet, DDGS based diets, digestibility

484P In ovo administration of *Lactobacillus plantarum* decreases in vitro *Salmonella enteritidis* population in cecal samples of chicks fed a wheat-soy diet. L. Macalintal*, L. Nolan, P. Glenney, A. Pescatore, T. Ao, M. Ford, and K. Dawson, *Alltech-University of Kentucky Nutrition Alliance, Lexington, KY.*

Our previous research demonstrated a reduction of the *Salmonella enteritidis* (SE) population in cecal samples of broiler chicks that were injected in ovo with *Lactobacillus plantarum* (LP; B4496; USDA) at 18d of incubation and fed a corn-soy based diet after hatch. For the current trial, in ovo administration of LP was conducted to evaluate its ability to suppress in vitro SE challenge using cecal samples harvested from broiler chicks fed a wheat-based diet. A total of 160 fertile broiler eggs at d18 of incubation were injected with 10^6 colony forming units (cfu) of LP suspended in 100ul MRS (de Man, Rogosa and Sharpe) broth. Non-injected and MRS broth-injected eggs were incubated along with the LP-injected eggs to evaluate hatchability. One-way ANOVA with means separated using Fisher's protected LSD was used to analyze the results. No difference was observed on hatch of fertile eggs (range 87–89.66%). At hatch, chicks were segregated into either LP or non-LP injected groups, then randomly allocated to 8 replicates pens/treatment with 20 chicks/pen. Dietary treatments did not affect production performance nor ileal digesta viscosity. Differences in jejunum morphology were observed at d0 but not d3. Longer villi ($P < 0.006$) and higher villus height:crypt depth ratio ($P < 0.033$) were observed in birds that received in ovo LP injection compared with non-LP injected group at day of hatch. At 3d post hatch, composite bacterial suspensions from the ceca of 3 birds from each of the 2 treatment groups were aseptically prepared in triplicate in McDougall's buffer (10% cecal content by volume) under anaerobic conditions. Ten ml of each cell suspension were added to separate serum bottles containing 1 g of layer feed as a substrate under an oxygen-free gas phase. To provide the pathogen challenge, each serum bottle was inoculated with a SE broth culture containing approximately 5.5 $x10^5$ cfu/ml. The SE in each bottle were enumerated after serial dilution on Brilliant Green Agar spread plates after 24h at 37°C. Cultured cecal samples harvested from birds receiving in ovo LP showed significant reduction in the total salmonella count compared with birds that did not receive LP in ovo ($P < 0.001$). The log concentration of SE in the LP cecal samples was reduced to 9.33 compared with 10.01. From this study, in ovo LP injection resulted in better intestinal cell morphology at hatch. Cecal bacterial suspensions from birds that had received live lactobacillus inoculation during the late incubation period then fed a wheat-soy diet during post hatch growth significantly suppressed the challenge SE.

Key Words: in ovo, lactic acid bacteria, probiotic, salmonella, villi

485P Effects of supplementing organic zinc and microalgae in broiler breeder diets on production performance and immune responses of progeny chicks fed a basal diet. L. Macalintal*, M. Paul, A. Pescatore, T. Ao, L. Nolan, M. Ford, and K. Dawson, *Alltech-University of Kentucky Nutrition Research Alliance, Lexington, KY.*

This study was conducted to investigate the effects of maternal dietary zinc (Zn) source and microalgae supplementation on growth performance and immune responses of progeny chicks fed a basal diet during post-hatch development. Maternal dietary treatments were arranged in a 2x2 factorial treatment structure with Zn oxide or BioplexZn as Zn sources and 2 levels of microalgae at 0 or 2%. The microalgae supplement, All-G-Rich, consisted of an *Aurantiochytrium limacinum*biomass (AURA; CCAP 4087/2; Alltech Inc., KY) containing 70% crude fat and 17% DHA.Hatched chicks from each of the maternal diets were allocated to 4 replicate cages with 6 chicks/cage. All chicks were fed a corn-soy-based diet through 21d. Chicks were raised in an environmentally controlled room with feed and water given on an *ad libitum* basis. Body weight, feed intake and relative lymphoid organ weights were recorded at 7, 14 and 21d. Immune responses were measured using hemagglutination antibody titers against intravenous injection of heterologous erythrocytes, 7% sheep red blood cells (SRBC) in phosphate buffer saline (PBS), and cutaneous basophil hypersensitivity responses as elicited by intradermal toe web injection of 100ug phytohemagglunin-P (PHA-P) in 100ul PBS. The SRBC injection was administered at d3 and d16 and PHA-P inoculated at d19. Data were analyzed using GLM for factorial analysis and mean differences were separated using Fisher's protected LSD set at $P < 0.05$. Maternal dietary treatment did not affect chick body weight gain and feed conversion at d21 ($P > 0.05$). There was a significant main effect of Zn source at d7 of age on weight and growth index for bursa. The bursa weight was reduced in chicks from hens whose diets were supplemented with BioplexZn compared with those chicks from hens fed Zn oxide. Relative bursal weight was increased by the addition of 2% algae at d14 and d21. In vivoT-cellular response was elevated on chicks hatched from hens supplemented with BioplexZn (Zn source main effect, $P < 0.05$). Increased thickness in the interdigital toe web after 48h post PHA-P injection was observed in chicks whose maternal diets contained BioplexZn. In vivohumoral immune response raised against SRBC as detected by the antibody titer during the primary immune response reaction was not different between the treatment groups but was higher for chicks hatched from non-algae supplemented hens during the secondary response. Results from this study indicate that maternally derived organic Zn in the form of BioplexZn can modulate certain immune responses through in vivo T-cell mediated immune responsiveness in progeny.

Key Words: organic zinc, algae, SRBC, PHA-P, lymphoid organs

486P Effect of different nutritional strategies on the incidence of pectoral myopathies in broiler chickens. A. Fonseca, C. Granghelli, C. Araujo, B. Leite, F. Roque, M. Dias, P. Pelissary, and L. Araujo*, *University of Sao Paulo, Pirassununga, São Paulo, Brazil.*

The objective of this study was to evaluate the effect of different nutritional strategies on the incidence of pectoral myopathy in broilers. The nutritional levels of the experimental diets followed the recommendations of Rostagno et al. (2017) for broilers with regular performance and for superior performance. A total of 1,755 male broilers from a commercial line of broiler chickens, distributed in a completely randomized design in 5 treatments (T1 - Control treatment: regular performance + 500 FTU of phytase; T2 - superior performance + 500 FTU of phytase; T3 - superior performance + 1500 FTU; T4 - superior performance + 500 FTU + antioxidant complex - vitamin E, C, organic selenium and organic zinc; T5 - superior performance + 1500 FTU, antioxidant complex - vitamin E, C, organic selenium and organic zinc) with 27 replications per treatment. The birds were distributed in 135 boxes, with 13 birds/replication. The antioxidant complex was included in the respective experimental treatments at the level of 0.175% containing 300 ppm of Vit E, 200 ppm Vit C, 200 ppm of zinc, and 0.4 ppm of selenium. At 49 d, 3 birds per replicate were slaughtered for evaluation of pectoral myopathy in broiler chickens. The data analysis was one way (ANOVA) using a completely randomized design and tested for significance between the dietary treatments means employing Tukey's (SAS, 2015). No significant effects ($P = 0.1975$) were found between treatments for the incidence of white striping (31.43%; 29.63%; 28.15%; 27.04%; 32.22%, respectively). However, broilers fed T4 treatment had reduced incidence of the wooden breast by 24% ($P = 0.0269$) when compared with the control treatment (32,57% × 42,86%). The results demonstrate that the supplementation of 500 FTU phytase and 0.18% of the antioxidant complex helps in reducing the incidence of pectoral myopathy of broiler chickens.

Key Words: wooden breast, white striping, vitamins, minerals, phytase

487P Visceral organ weight and indices of gastrointestinal ecology in Lohmann Select Leghorn and Shavers Heritage hens fed high fiber diets. A. W.-Van Humbeck* and E. Kiarie, *University of Guelph, Guelph, ON, Canada.*

Fiber can be used as a strategy to stimulate gastrointestinal ecology and physiology. However, there is limited literature in regard to feeding high fiber to laying hens and the role of processing fibrous feedstuffs. Furthermore, comparative effects in modern strains and their genetic predecessors are not well defined. Therefore, the present study evaluated 3 diets consisting of a commercial mash, or a mix of commercial mash and ground (GOH) or unground (UGOH) oat hulls at a ratio of 80:20 wt/wt, when fed to 57-wk Lohmann Select Leghorn- Lites (LSL) and 44-wk Shavers Heritage White Leghorns (SHWL). Mean particle size and SD of the control, GOH, and UGOH feed was 267 ± 11.7, 277 ± 6.1 and 276 ± 8.0μm, respectively. A total of 288 birds were placed by breed (6 hens/cage) and based on 1-wk egg production, allocated to diets to give 8 replicates. Feed and water was given ad libitum throughout the 28- d trial. Excreta samples were collected on d 25–28 and subsequently frozen at −20°C for future analyses. At the end of the trial, 2 birds per cage were weighed and sacrificed for liver and jejunal tissue with remaining birds sacrificed to access cecal digesta for short-chain fatty acid (SCFA) determination. Data was subjected to 2-way ANOVA in JMP 13.1 with fixed effects of diet, breed and their 2-way interaction. An interaction ($P < 0.05$) was seen on total SCFA such that LSL hens fed fiber had a lower total SCFA concentration than LSL hens fed control, or SHWL fed any diet. In general, LSL had lower SCFA concentrations than SHWL (101 vs. 127 μmol/g, $P < 0.01$), with SHWL exhibiting lower propionic ($P < 0.01$), but higher iso-butyric ($P < 0.01$) and n-butyric acid concentrations than LSL ($P < 0.01$). The interaction ($P < 0.05$) and diet effect ($P = 0.003$) seen on total SCFA can mainly be attributed to lower acetic acid production ($P = 0.015$) in both breeds when fed high fiber. Breed effects ($P = 0.011$) were also seen on crypt depth (CD) but not on villi height (VH), such that CD was higher in SHWL, contributing to a lower ($P < 0.01$) VH: CD ratio. In addition, although LSL trended ($P = 0.08$) to have a heavier wet liver weight than SHWL, the dry liver wt did not differ ($P > 0.05$). An effect of diet ($P < 0.01$) was seen on

excreta DM such that high fiber feeds had higher DM% regardless of processing, with a breed effect ($P < 0.01$) of higher DM% in LSL. To conclude, modern birds with the help of genetic advancements, can be seen to have higher indices aiding in absorption regardless of diet, however high fiber feedstuffs with or without processing may impact gastrointestinal health through alteration of SCFA production, intestinal morphology as well as fecal moisture.

Key Words: layer, fiber, oat hulls, liver, gastrointestinal health

488P Effect of super-dosing of phytase, calcium, and phosphorus concentration on broiler performance and bone density. C. Araujo*, R. Marangoni, L. Araujo, C. Granghelli, S. Silva, M. Cuadros, P. Zorzetto, and Y. Sartore, *University of Sao Paulo, Pirassununga, São Paulo, Brazil.*

This trial was conducted with the objective of evaluating the reduction of calcium (Ca) and available phosphorus (Pa) for broilers fed diets containing increased levels of phytase. Treatments were arranged in a 3 × 4 completely randomized factorial design varying in Ca and Pa (100%, 90%, and 80%) and phytase (0; 500; 1,000; and 1,500 FTU/kg). One thousand and one hundred and 52 male chicks Cobb-500 were randomly allocated to 12 treatments with 8 replicates having 12 broilers each. The diets were corn and soybean meal based and were formulated based on Rostagno et al. (2011). The data analysis was one way (ANOVA) using a completely randomized design and tested for significance between the dietary treatments means employing Tukey's (SAS). Performance traits, carcass yield, and commercial cuts were evaluated. Furthermore, a bone quality study, with the analysis of tibia density, was performed. A significant interaction between factors studied was observed in which the addition of phytase promoted a linear increase in body weight gain for diets with 90% and 80% of Ca and Pa at 21 d. In addition, the increased levels of phytase in the diets reduced linearly the feed: gain ratio at 21 d. Overall performance (1–42 d), showed that phytase supplementation (500 and 1500 FTU) improved body weight gain and feed: gain ratio. Also, treatments had no effect on carcass yield, commercial cuts, and tibia density. In summary, the findings of this study suggested that phytase supplementation improved growth performance and it was possible to use low Ca and Pa concentration without affecting bone quality and performance.

Key Words: bone quality, carcass yield, enzyme, minerals, performance

489P Effect of varying dietary methionine and cysteine concentrations on growth performance of the French guinea fowl broiler. S. Nahashon*, C. Richards, T. Bowden-Taylor, B. Kimathi, and C. Khwatenge, *Tennessee State University, Nashville, TN.*

This study was undertaken to assess dietary methionine and cysteine concentrations for optimum growth performance of the French guinea fowl broiler. In a 3 × 3 factorial arrangement, 405 1-d-old French guinea keets were randomly assigned to experimental diets containing 3 concentrations of methionine (0.50, 0.45, and 0.40%) each in combination with 3 concentrations of cysteine (0.35, 0.40, 0.45%). The diets were isocaloric (3,100 kcal ME/kg) and isonitrogenous (21% CP) and were fed from hatch to 8 weeks of age (WOA). Each dietary treatment was replicated 3 times with 15 birds per replicate, and both feed and water were provided *ad libitum.* A 23-h light regimen was provided to the birds for the 8 weeks. The experimental birds were evaluated for body weight gain (BWG), feed consumption (FC), and feed conversion ratios (FCR) to determine optimal dietary methionine and cysteine. Data were analyzed using Two-way ANOVA option of the GLM procedure of SAS. At 0–4 WOA, birds that were fed diets supplemented with 0.50% methionine and 0.40% cysteine had higher BWG and FC ($P < 0.05$) than those fed other dietary treatments. However at 5–8 WOA, birds fed diets containing 0.45% methionine and 0.4% cysteine exhibited higher BWG and FC when compared with other dietary treatments. The FCR was significantly lower ($P < 0.05$) in birds fed diets containing 0.45% of each methionine and cysteine at 0–8 WOA. Correlations between both methionine and cysteine, and BWG and FC were positive and significant, while those between methionine and cysteine, and FCR were negative. Therefore, at 0–4 WOA, methionine and cysteine will be optimally utilized by the French guinea broilers at 0.50% and 0.40%, respectively. However, at 5–8 WOA, the optimal dietary methionine and cysteine for these birds seem to be 0.45%.

Key Words: French guinea fowl, methionine, cysteine, growth performance

490P Growth responses of 10-d-old Ross 308 broilers fed diets with different levels of amino acids. Y. K. Sung*[1], S. H. An[2], and C. Kong[2], *[1]Easy Bio. Inc., Seoul, Korea, [2]Kyungpook National University, Sangju, Korea.*

The objective of the present study was to compare growth responses of 10-d-old male broiler chickens (Ross 308) fed diets formulated with different dietary levels of Lys, Met, and Thr. A total of 960 birds (37.6g ± 2.42) were allocated to 4 treatments in a randomized complete block design with 12 replicate cages per treatment. The 4 experimental diets were formulated based on corn, soybean meal and synthetic amino acids to contain different levels of Lys, Met, and Thr based on NRC (1.06, 0.49, and 0.80%) recommendations, Evonik ideal protein (1.20, 0.50, and 0.82%), the Test levels 1 (1.29, 0.59, and 0.91%), and the Test levels 2 (1.21, 0.38, and 0.75%), respectively. L-glutamic acid was used to make all diets equal in crude protein at 21.9% of the diet. The Test levels 1 and 2 were acquired from analyses of amino acid in commercial diets which were collected from South Korea. The data for average daily gain (ADG) and gain to feed ratio (G:F) were analyzed by using a general linear model procedure in SAS. The birds fed the diet based on NRC recommendation showed the least (12.79 g/kg, $P < 0.05$) ADG while there was no difference among other treatments. The least G:F was observed from birds fed the NRC recommendation diet (0.85, $P < 0.05$) followed by Test levels 1 (0.92), Test levels 2 (0.93), and Evonik ideal protein (0.94). In conclusion, the growth responses of broiler chickens at 10 d of age can be affected by different dietary levels of Lys, Met, and Thr.

Key Words: amino acid, broiler, ratio, growth performance

491P Nutrient-specific expression of proteins in the small intestine of broilers as shown by LC-MS/MS analysis. S. Kang[1], K. W. Lee[2], H. Chang[1], B. H. Moon[3], and Y.-H. Choi*[1], *[1]Gyeongsang National University, Jinju, Korea, [2]Konkuk University, Seoul, Korea, [3]Celltech Co., Ltd., Eumseong, Korea.*

Our previous study, using 2-dimensional electrophoresis with matrix assisted laser desorption/ionization time-of-flight-mass spectrometry (MS)/MS analysis, showed that the expression of proteins in the small intestine responded to a nutrient-oriented fashion. The primary objective of the current study was to continuously determine the effects of nutrients on protein expression in the small intestine of broilers using by LC-MS/MS analysis. At the end of animal experimentation, in which day-old broilers were randomly allocated into one of 3 dietary treatments with 7 pens each and 14 chicks per pen and provided for 5 weeks with one of

3 diets: control, arginine-, or conjugated linoleic acid (CLA)-rich diet, the small intestine were cut out into the 3 different anatomical sections: duodenum, jejunum and ileum. Their length and weight were thereafter measured after being longitudinally cut open, gently wished with saline, and softly soaked up with paper towels. Each of 3 intestines was then folded at their midpoint and was cut out so that their proximal half (3 cm long) were frozen on dry ice and stored at −80°C. Protein was extracted from duodenal tissues using a TCA/acetone/phenol extract method, with which one-dimensional SDS-PAGE was run to confirm the quality of proteins. The bands were cut out and digested by trypsin. The peptide samples obtained were subjected to nano-LC-MS/MS analyses using an Eksigent nano-LC 415 system connected to a TripleTOF6600 mass spectrometry system with a nanoelectrospray ion source. The data files obtained were processed using UniProt and Protein Pilot 5.0.1 (SCIEX, Redwood City, CA, USA) database software, and curated to yield the protein identifications using 1% global false discovery rate (FDR) determined by the in-built FDR tool within ProteinPilot software. The results were validated by using Scaffold and perSPECtive (Proteome Software Inc., Portland, OR). The initial results of the analysis showed that a total of 828 proteins were identified, of which 381 proteins were uniquely counted as nutrient-specifically responding proteins. Among them, 55 proteins were upregulated either in Arg- or CLA- rich dietary group while none of them were counted in control. On the other hand, 24 proteins were highly responded compared with either Arg- or CLA- rich diet. The results of the current study, taken together with our previous findings, suggest that dietary nutrients play a key role in the regulation of intestinal protein expression in broilers.

Key Words: LC-MS/MS, nutrient, protein experssion, intestine

492P Energy utilization in soybean meal and corn samples for organic broiler production. H. Leung*[1] and E. Kiarie[2], [1]*University of Guelph, Guelph, ON, Canada.*

Organic broiler production require use of certified organic feedstuffs for growing husbandry practices and feed processing. Feedstuffs that are also non-GMO can be used to add value. However, little is known of how the nutritive value of these feedstuffs compare with conventional feedstuffs. The objective of this study was to determine energy utilization in organic non-GMO soybean meal (SBM) and corn used by Ontario organic broiler producers. Samples of organic non-GMO SBM and corn were procured from a local certified organic broiler feed manufacturer. One sample of each feedstuff was either locally produced or imported. Conventional corn and SBM samples were sourced from commodity markets for comparison. A total of 560 d old male broiler chicks (Cobb 500) were placed in 56 cages and fed a commercial organic starter diet until d 13. On d 14, birds were weighed and allocated to cages (10 birds/cage; n = 8). They were allocated to one of 7 semi-purified wheat starch-based diets: 1) conventional SBM (CSBM), 2) local organic SBM (OSBML), 3) imported organic SBM (OSBMI), 4) conventional corn (CC), 5) local organic corn (OCL), 6) imported organic corn (OCI) and 7) N free wheat starch (NFD). The SBM diets were formulated to contain ~18% protein and corn diets were formulated to contain 90% corn. All diets had Titaniunm dioxide as an indigestible marker and ratio of wheat starch to sucrose and soy oil was identical to NFD to calculate AME by difference method. Birds were given feed and water *ad lib*. Excreta samples were taken on d 17–20. On d 21, all birds were sacrificed for ileal digesta and 2 of the birds had pancreas weights taken. The concentration of GE in CSBM, OSBML, OSBMI, CC, OCL and OCI was 4,653, 5,054, 4,923, 4,514, 4,528 and 4,5001 kcal/kg DM, respectively. Corresponding concentrations of CP and crude fat on %DM were 48.6 and 1.0, 50 and 7.1, 44.7 and 6.03, 9.8 and 5.7, 7.7 and 1.8, and 7.6 and 2.5, respectively. Pancreas weight increased (P = 0.02) by 18% in birds fed OSBML compared with CSBM. The apparent retention of CP was 24% higher (P = 0.007) in OSBML compared with the other SBM samples. The AMEn differed (P < 0.001) across all samples of SBM, with the highest in OSBML, followed by OSBMI and then CSBM (3119 vs 2771 vs 2531 kcal/kg DM). The AMEn of CC was higher (P < 0.01) than that of organic corn samples, the values were 3508, 3287 and 3363 kcal/kg DM for CC, OCL and OCI, respectively. In conclusion, organic SBM samples had higher residual oil reflective of processing procedures and as result higher AMEn relative to CSBM samples, however, increase in pancreas weight was indicative of higher concentration of residual trypsin inhibitors. The CC had higher AMEn relative to organic corn samples.

Key Words: organic broiler, soybean meal, corn, energy utilization

493P Supplementation of microalgae in pre-starter diet improves early growth performance and bone strength of broiler chicks. M. Paul*, T. Ao, A. Pescatore, M. Ford, and K. Dawson, *Alltech-University of Kentucky Nutrition Research Alliance, Lexington, KY.*

All-G-Rich is a microalgae *(Aurantiochytrum limacinum* CCAP 4087/2; Alltech, Inc.) that contains 17% docosahexaenoic acid (DHA). A trial was conducted to evaluate the effects of dietary supplementation of All-G-Rich in a pre-starter diet on broiler growth performance and bone strength. Seven replicate pens of 24 chicks each were randomly assigned to 7 dietary treatments. A control diet consisted of feeding a corn-soybean meal basal diet with no supplementation of All-G-Rich. The remaining 6 treatments consisted of feeding the basal diet supplemented with 1, 0.7 or 0.5% All-G-Rich for either 1–10d or 1–18d. Bone breaking strength was assayed at d 18. Data were analyzed using one-way ANOVA (SAS, v9.4). Dietary supplementation of All-G-Rich did not affect the overall growth performance during the 42-d study. However, at 10 d of age, chicks fed the diet containing 1% All-G-Rich had higher (P < 0.05) weight gain compared with the chicks fed the control diet or diet with 0.7% All-G-Rich. Chicks fed diets containing 0.5% or 0.7% All-G-Rich had higher (P < 0.05) feed intake compared with those fed control diet at 10 d of age. The femur breaking strength of chicks fed diets with 0.5 or 1% All-G-Rich for 18 d was higher (P < 0.05) than that of chicks fed the control diet or the diets with 0.5% All-G-Rich for 10 d or 0.7% All-G-Rich for 18 d. The femur breaking strength of chicks fed diets with 1% All-G-Rich for 10 d was higher (P < 0.05) than that of chicks fed diet with 0.7% All-G-Rich for 18 d. These results indicate that dietary supplementation of All-G-Rich in broiler pre-starter diet could positively impact the early growth performance and bone quality of broiler chicks.

Key Words: microalgae, DHA, growth performance, bone strength, broiler chicks

494P The effect of *Tenebrio molitor* oil on the growth performance, nutrients digestibility and selected blood parameters of broiler chicken. A. Benzertiha*[1], D. Józefiak[1], B. Kieronczyk[1], M. Rawski[1], S. Talibov[1], S. Nogales-Merida[1], P. Gobbi[2], A. Józefiak[1], and J. Mazurkiewicz[1], [1]*Poznan University of Life Sciences, Poznan, Poland,* [2]*HiProMine S.A., Robakowo, Poland.*

The study was conducted to evaluate the effect of total replacement of palm oil and poultry fat with *Tenebrio molitor* oil in broiler chicken diet on the growth performance, GIT pH, nutrient digestibility different blood parameters and internal organs weight. A total of 72 7-d-old

female Ross 308 were used. The birds were randomly distributed to 3 different groups, 12 replicates per group and 2 birds per replicate. The experiment was 30 d in metabolic cages (40 × 40 × 40 cm). The basal diet was formulated on maize and soybean meal basis. 5% of palm oil, poultry fat or *Tenebrio molitor* oil was added to the diet. The body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were measured during d 7, 14, 21 and 30. Samples for apparent ileal digestibility were collected and pH of crop, jejunum, and ceca was measured. Ten birds per replication per group were randomly selected and blood samples were collected from the wing vein The nonesterified fatty acid (NEFA), glucose, triglycerides (TG), cholesterol, total protein, and albumin. The weight of liver and pancreas was measured and the organs weights in relation to body weight (BW) (% of BW) were calculated. Data were tested using the General Linear Models (GLM) procedure of SAS software. In the experiment, means were separated using Duncans' tests following one-way ANOVA. In all periods of the experiments, the application of *Tenebrio molitor* oil did not have any statistically different effects on the BWG, FI or FCR in comparison to the treatments with poultry fat or palm oil. The pH value of the GIT content did not differ significantly among all treatments ($P = 0.25$, $P = 0.98$, $P = 0.72$ respectively). The value of apparent ileal digestibility of crude protein, ether extract, and apparent metabolizable energy did not show any significant differences among all treatments ($P = 0.31$, $P = 0.36$, $P = 0.24$ respectively). No statically significant differences were observed on the NEFA, glucose, total protein, and cholesterol. However, the use of *Tenebrio molitor* oil significantly decreased the level of triglyceride in comparison to poultry fat and palm oil ($P = 0.04$). Statistical significant differences were observed in the liver ($P = 0.03$) weight, where the lowest value was observed in *Tenebrio molitor* oil treatment. While no statistical differences were observed on the pancreas involvement in the body weight. in conclusion, the use of *Tenebrio molitor* oil in broiler chicken diet did not show any effect on the growth performance neither the ileal nutrient digestibility in comparison to poultry fat or palm oil. While *Tenebrio molitor* oil reduced the level of triglyceride in blood serum as well as the liver weight in relation to the live body weight.

Key Words: insects, *Tenebrio molitor*, broiler chicken, palm oil, poultry fat

495P Correlation of feed ingredient characteristics with flowability as measured by angle of repose. O. Wedegaertner*, A. Fahrenholz, and P. Ferket, *North Carolina State University, Raleigh, NC.*

Feed ingredient flowability is an important handling characteristic that affects the efficiency of feed manufacturing depending on equipment design. Flowability can be estimated by the angle of repose (AOR), but this estimate may be significantly influenced by other physical characteristics of the feed ingredient sample. We hypothesize that hygroscopicity, bulk density, compressibility, particle size (Dgw), and particle size variability (Sgw) are characteristics of feed ingredients that influence flowability. The objective of this study was to identify the most significant handling characteristics that influence AOR so they can be controlled in test protocols. Triplicate samples of 26 standard broiler feed ingredients were analyzed for AOR, hygroscopicity, Dgw, Sgw, bulk density, tapped density, and Carr's compressibility index. Correlation values between AOR and each characteristic were statistically determined by multivariate analysis (JMP Pro 13, SAS Institute, Cary NC). No significant correlation was observed between AOR and hygroscopicity, bulk density, tapped density or Dgw, indicating that these physical characteristics alone have minimal affect on ingredient flowability. Correlation between AOR and Sgw ($R^2 = 0.56$) was significant ($P \leq 0.01$) as well as correlation between AOR and compressibility ($R^2 = 0.60$, $P \leq 0.01$). Sgw and the compressibility of an ingredient have a greater influence on AOR than density, hygroscopicity or mean particle size. There was also a statistically significant correlation between compressibility and Sgw ($R^2 = 0.44$, $P \leq 0.05$), indicating that Sgw is influential on an ingredient's compressibility. Ingredients were grouped into categories based on Dgw (76–183, 183–240, 240–471, 471–802 and 802–1210 μm) and Sgw (1.08–1.62, 1.62–1.90, 1.90–2.29, 2.29–2.52 and 2.52–3.97 μm) and the correlation between AOR and compressibility of ingredients within each group was then calculated. Ingredients categorized in the small Dgw groups (76–183, 183–240, and 240–471 μm) and small Sgw groups (1.08–1.62 and 1.62–1.90 μm) had the most significant correlation between AOR and compressibility [($R^2 = 0.87$, $R^2 = 0.96$, and $R^2 = 0.94$, $P \leq 0.1$) and ($R^2 = 0.72$ and $R^2 = 0.98$, $P \leq 0.1$) respectively]. In contrast, AOR and ingredient compressibility are not significantly correlated in the larger Dgw and Sgw groups. Compressibility and Sgw have the greatest influence on AOR, whereas density, hygroscopicity and Dgw have no effect on AOR. Ingredients with small particle size (Dgw ≤ 471 μm) and less particle size variability (Sgw ≤ 1.9 μm) have a stronger correlation ($R^2 > 0.7$, $P \leq 0.1$) between AOR and compressibility than ingredients with a large Dgw and large Sgw.

Key Words: feed ingredients, flowability, angle of repose, compressibility, particle size

496P Determining the effects of feeding two broiler strains varied crumble size and intact pellets (d 0-18) on gastrointestinal measurements. A. Ruzicka*[1], M. Lemons[1], C. Ennis[1], C. McDaniel[1], J. Moritz[2], and K. Wamsley[1], *[1]Mississippi State University, Mississippi State, MS, [2]West Virginia University, Morgantown, WV.*

Our laboratory found that feeding an average crumble (C) particle size of 2200 to 3736 μm improved starter performance. To explain these benefits, gastrointestinal samples (GI) were collected at the end of the study (d 15). However, no clear connection was made with this trial as only one sampling period was performed. Therefore, the current study's objective was to evaluate the effects of feed form (FF) and feed quality (FQ) fed in the starter phase when fed to 2 strains (S; SA or SB) on GI development at 3, 5, 7, 10, 14, and 18 d of growth. This study employed a 2 (S) × 2 (FF) × 3 (FQ) factorial arrangement within a RCBD. Crumbles or intact pellets (IP) were fed as one of 3 different FQ. These FQ included: Low-2210 μm (C) or 40% IP, Medium (Med)-3010 μm (C) or 60% IP, and High-3388 μm (C) or 80% IP. One nutritionally common diet was manufactured at a commercial mill with modifications performed to adjust desired FQ for each FF. On d 3, 5, 7, 10, 14, and 18, one bird/pen was randomly selected for GI measurements. Birds were weighed for organ weights to be placed on yield relative to BW basis (YD). Due to the complexity of the design and space limitations, only key findings will be presented in this abstract. On d 3 and 7, duodenum YD (DYD) was affected by FF × FQ ($P < 0.05$). For both sampling d, birds fed IP of low FQ consistently resulted in the highest DYD, but were similar to Med FQ for both FF. However, at d 3, C of High FQ resulted in the lowest DYD; with IP of High FQ performing intermediate. For d 7, High FQ of both FF resulted in intermediate DYD, while feeding C of low FF resulted in the lowest DYD. At d 5 and 10, S × FQ affected jejunum YD (JYD) (both $P = 0.05$). Looking at d 5, both strains resulted in similar JYD for Med and High FQ; with SB resulting in a higher JYD if fed Low FQ. At d 10, both S resulted in similar JYD if fed Low and High FQ, with feeding Med FQ increasing JYD for broilers of SA. At d 18, feeding IP, regardless of FQ and S, decreased JYD ($P = 0.07$). At d 18, a FF × FQ interaction ($P = 0.04$) demonstrated that feeding birds C of Low FQ increased ileum YD (IYD), with birds fed IP of Low FQ decreasing IYD. Birds fed Med and High FQ resulted in similar and

intermediate IYD, regardless of FF. At d 10, S × FQ interactively affected yolk sac weight (YSW; $P = 0.002$). Both S had a decreased YSW when fed Low and Med FQ. However, High FQ fed to SB resulted in the highest YSW, whereas feeding high FQ to SA birds decreased YSW to that similar of the other FQ and S. These data demonstrate that the relationship between FQ × FF × S is complex; warranting correlation of sampling data to live performance observed from the companion study ran in conjunction with the current study.

Key Words: pellet, crumble, feed form, feed quality, gastrointestinal tract

497P The impact of varying starter digestible lysine and energy levels on male Cobb MV × Cobb 500 broiler 42 day growth performance and processing parameters. R. Hirai*[1], L. Mejia[2], C. Coto[2], J. Caldas[2], C. McDaniel[1], and K. Wamsley[1], *[1]Mississippi State University, Starkville, MS, [2]Cobb-Vantress Inc., Siloam Springs, AR.*

In 2017, Cobb introduced a new broiler breeder product, the MV male, with goals to improve FCR, decrease mortality, and maintain fertility in comparison to the Cobb MX male. The objective of the current study was to evaluate the impact of feeding varying digestible lysine (dLys) and energy (AME) levels from 0 to 14 d on performance and processing yields of 42 d old Cobb MV × Cobb 500 male broilers. A total of 1,344 male chicks were obtained from a commercial hatchery. There were 8 treatments (12 replicate floor pens) with 14 chicks/pen (0.08 m^2/bird). Starter diets from d 0–14 were formulated to contain dLys of either 1.18% or 1.28%; and AME levels of 2,890; 2,980; 3,070; and 3,160 kcal/kg. Common grower and finisher diets were provided for the remainder of the grow-out from d 15–42. This study utilized a RCB design containing a 2 (dLys) × 4 (AME) factorial arrangement of treatments with d 0 BW as a covariant and Tukey's range test for mean separation. Measured variables were BW, BW gain (BWG), feed conversion ratio (FCR) corrected and uncorrected for mortality (uFCR), % mortality, and d 42 processing data (3 birds/pen). For d 0–7, no difference was observed for any performance metric ($P > 0.05$). On d 0–14, birds receiving 1.18% dLys had a higher FCR and uFCR ($P < 0.001$). In addition, birds receiving 2,890 kcal/kg AME diets had the highest d 0–14 FCR when compared with birds fed other AME levels. Feeding 2,980 kcal/kg AME diets resulted in birds with higher d 0–14 FCR when compared with those fed 3,070 and 3,160 kcal/kg AME diets ($P < 0.001$). For d 0–28, birds fed starter diets containing 2,890 kcal/kg AME had the highest FCR (by ~2 points) as compared with birds fed diets formulated to AME of 3,070 kcal/kg and 3160 kcal/kg in the starter; those fed starter diets containing 2,980 kcal/lb AME performed intermediately ($P < 0.001$). Additionally, a dLys × AME interaction was observed for d 0–28 uFCR ($P = 0.023$). Increasing starter AME levels from 2,890 to 2,980 kcal/kg improved d 0–28 uFCR, regardless of starter dLys levels. Feeding higher starter AME levels (3,070 or 3,160 kcal/kg) in conjunction with 1.18% starter dLys level improved 0–28 d uFCR when compared with birds fed 1.28% starter dLys. However, overall growth performance data (d 0–42) and processing data did not demonstrate any dLys × AME interactions nor significance for the main effects. It must be noted that the current study's results may be different if other formulation metrics were utilized in the grower and finisher phases. Thus, further research should investigate other formulation strategies during these growth phases and also evaluate the effects of different dLys and AME levels on female Cobb MV × Cobb 500 broilers during various feeding phases.

Key Words: new commercial broiler cross, digestible lysine, AME, growth performance, processing yield

498P Irradiated flax seed versus adding enzyme in flax seed diets increased performance and n-3 fatty acids of chicken meats. M. H. B. Moghadam* and M. Nayefi, *Sari Agricultural Sciences and Natural Resources University, Sari, Mazandaran, Iran.*

Flaxseed (FS) is the greatest source of linolenic acid (18:3n-3; LNA) of oilseed. However, flaxseed contains some anti-nutritional factors that had been reported to decrease performance, nutrient utilization, and tissue n-3 FA incorporation of birds depending on the dietary levels used in the diet. Radiation is a method that known to reduce anti nutritional factors effectively and upgrade the nutritional quality of plant-origin feeds. It is hypothesized that in compare of adding enzyme, radiation is used for improvement nutritive value of seeds in the diet of broilers for effective modification of performance, carcass characteristics, digesta viscosity and meat fatty acid (FA) in broiler chicks. In a completely randomized design, 320 d-old broiler chicks were assigned to the 5 diets with 4 replicates (16 birds per each) for 42 d. Dietary treatments were Control, RFS (%10 Irradiated Flax seed, 1–42 d), RFSG (diet containing %10 Irradiated flaxseed for the grower period,11–23 d), RFSF (diet containing %10 Irradiated flax seed for the finisher period, 24–42 d), FSE (diet containing %10 flaxseed plus Enzyme, 1–42 d), and FSW (diet containing %10 flax seed without Irradiation or adding enzyme, 1–42 d). At 42 d of age, 2 birds per pen randomly selected for the determination of carcass characteristics, digesta viscosity and FA profile of thighs and breasts. The RFS diet increased ($P < 0.05$) body weight gain (BWG) of broilers compared with the other diets. In all periods, feed conversion ratio (FCR) decreased significantly ($P < 0.05$) in broilers consumed RFSG and RFSF diets compare with other treatments. Birds fed RFS increased ($P < 0.05$) relative weight of thighs muscle compared with other groups. Radiation and enzyme were not significant effect on digesta viscosity, and tibia bone parameter. No effect of treatments observed on DHA (docosahexaenoic acid), EPA (eicosapentaenoic acid), total monounsaturated fatty acids (MUFA), total saturated fatty acids (SFA), and total lipids in breast muscle. However, the RFS significant increased total n-3 FA among the treatments; in addition, the rate of n-6/n-3 decreased in RFS than FSE in breasts muscles. The effect of total n-3 fatty acids for thighs were significant ($P < 0.05$) among the treatments which were 14.10, 4.64, 8.96, 10.33, 9.96 in the RFS, RFSG, RFSF, FSE, FSW, respectively. The SFA and n-6/n-3 decreased ($P < 0.05$) in RFS compare with other diets. Total lipid content of thighs significant increased ($P < 0.05$) in FSW compare with other treatments. These results indicate that in comparison of Irradiation and enzyme, Irradiation causes increased n-3 FA in thighs and breasts and improved performance in broilers.

Key Words: DHA, EPA, flax, broiler, radiation

499P Effects of corn particle size during the finisher period on broiler growth performance. A. Rubio*, A. Lopez, J. Hess, W. Berry, W. Dozier, III, and W. Pacheco, *Auburn University, Auburn, AL.*

The poultry industry grinds corn to approximately 800 µm before its incorporation into broiler diets in the starter, grower, and finisher periods. However, feeding coarse particles (>1,000 µm) has been reported to stimulate gizzard development in broilers and reduce energy consumption during the grinding process. Therefore, the ability of maturing birds to tolerate coarser feed particles may improve growth performance

and generate economical revenues in the manufacturing process. The objective of this study was to evaluate the effects of corn particle size on broiler live performance from 28 to 42 d of age. One thousand 1-d old male Ross × Ross 708 broiler chicks were randomly distributed among 4 treatments with 10 replicate pens per treatment and 25 birds per pen. The treatments consisted of 4 corn particle sizes (615, 863, 1,644, and 2,613 µm). Corn was ground using a roller mill using different roll gap widths to produce the 4 particle sizes. Geometric mean particle size by mass (d_{gw}) was determined according to the ASABE method S319.4 using a 13-sieve stack with US sieve numbers 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, 270, and pan. A Ro-Tap shaker was used to sift the samples for 10 min. Birds were raised on used litter that was top-dressed with new pine-shavings at the beginning of the study. Common crumbled starter and pelleted grower diets were offered from 1 to 14 and 15 to 28 d of age, respectively. Data were statistically evaluated using ANOVA procedure and means were separated by Tukey's honestly significant different procedure. Feed intake (FI), body weight (BW), and feed conversion (FCR) were determined at 14, 28 and 42 d of age. Particle size of corn did not influence body weight gain (BWG) and FCR of broilers from 28 to 42 d of age ($P \geq 0.05$). Broilers fed diets with 1,644 µm corn particle size had higher feed intake than birds fed diets with corn ground to 615 µm from 28 to 42 d of age ($P \leq 0.05$). The results of this experiment indicated that birds could be fed up to 2,613 µm during the finisher period without a negative effect on growth performance.

Key Words: coarse, corn, gizzard, grinding, performance

500P Effect of digestible lysine levels and metal-amino acid complexes on performance and breast meat characteristics in broilers. L. Linares*[1], M. Rebollo[1], A. Fireman[1], D. Neves[1], A. Grove[1], D. Bourassa[2], J. Hess[2], and W. Pacheco[2], *[1]Zinpro Corporation, Eden Prairie, MN, [2]Auburn University, Auburn, AL.*

One-day-old Ross 708 male broiler chicks (n = 1664) were used to evaluate performance, processing traits, blood parameters, and meat quality of 2 digestible Lysine (dLys) levels and 4 mineral treatments. Birds were fed in 4 phases: starter (1 to 13 d), grower (14 to 28 d), finisher (29 to 42 d), and withdrawal (43 to 56 d). Phytase was added to all diets at 1500 FTU/kg. During the grower phase birds were fed 100 or 85% recommended levels of dLys. Throughout the study birds were offered 1 of 4 mineral treatments: sulfates (100 ppm Zn from $ZnSO_4$ and 40 ppm Fe from $FeSO_4$); AvZn (40 ppm Zn from $ZnSO_4$ + 60 ppm Zn from Zn amino acid complex and 40 ppm Fe from $FeSO_4$); AvFe (100 ppm Zn from $ZnSO_4$ and 40 ppm Fe from Fe amino acid complex); AvZnFe (40 ppm Zn from $ZnSO_4$ + 60ppm Zn from Zn amino acid complex and 40 ppm Fe from Fe amino acid complex). Data was analyzed by JMP13 as randomized complete block design with a 2 × 4 factorial arrangement. Birds fed 85% dLys had decreased ($P < 0.001$) body weight gain (BWG) and increased feed conversion ratio (FCR) during the grower phase, but no differences in performance were observed between birds fed dLys treatments during the finisher or withdrawal phases. At 56 d, footpad lesion score 1 was lower ($P < 0.01$) for birds fed 85% dLys during the grower period. Birds fed AvFe and AvZnFe had improved ($P < 0.01$) BWG and FCR during the starter phase compared with birds fed sulfates (475, 478 and 455 g BWG for AvFe, AvZnFe, and sulfates, respectively and 1.17, 1.18, and 1.20 FCR for AvFe, AvZnFe, and sulfates, respectively). At 43 d, birds receiving 85% dLys had a 21% decrease in severity of wooden breast ($P = 0.01$) compared with birds fed 100% dLys and there was a dLys × mineral treatment interaction ($P < 0.05$) for breast yield. Within each dLys level, breast yield did not differ ($P > 0.10$) among mineral treatments; however, birds fed AvZn and AvFe mineral treatments with 100% dLys had greater ($P < 0.05$) breast yields compared with birds fed these mineral treatments and receiving 85% dLys (29.32 and 28.19 for AvZn birds fed 100 and 85% dLys, respectively and 29.03 vs 28.0 for AvFe birds fed 100 and 85% dLys, respectively). Birds fed AvFe and AvZnFe had increased ($P < 0.05$) blood hematocrit at 49 d of age compared with birds fed sulfates. Cooking losses and oxidative status (TBARS) of meat did not differ ($P > 0.10$) between dLys levels or among mineral treatments. Reducing dLys by 15% in grower diets reduced performance and impaired FCR during the grower phase, reduced breast yield and severity of wooden breast on 43 d. Inclusion of Zn and Fe amino acid complexes in diets improved performance during the starter phase and improved breast yield when recommended levels of dLys were fed.

Key Words: broiler, digestible lysine, iron, metal-amino acids, zinc

501P Effect of low dose organic trace mineral complex on production performance, egg quality and fecal mineral excretion of laying hens. J. Qiu[1], X. Lu[1], L. Ma[1], C. Hou[1], J. He[1], B. Liu[1], G. Lin[2], T. Ao*[3], and D. Yu[1], *[1]College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China, [2]Alltech Biological Products (Beijing) Co. Ltd., Beijing, China, [3]Center for Animal Nutrigenomics and Applied Animal Nutrition, Alltech Inc., Nicholasville, KY.*

Organic trace minerals have been shown to be more bioavailable than inorganic mineral salts and could be used at a lower rate in animal feed to minimize mineral excretion from animal waste that is a big concern in modern animal production. The objective of this study was to investigate the effect of low-dose organic trace mineral complex on production performance, egg quality, yolk and fecal mineral concentration of laying hens during the late laying period. A total of 405 healthy hens (HY-Line White, 52-week-old) with similar body weight and egg-laying rates were randomly divided into 3 treatments, with 9 replicates per treatment and 15 birds per replicate. The experiment lasted 56 d. Diets were arranged as follows: CON: a basal diet + inorganic Fe, Cu, Mn and Zn at 36, 12, 90 and 90 mg/kg (commercial level), respectively; ITM: a basal diet + inorganic minerals equivalent to 1/3 levels of CON; OTM: a basal diet + organic minerals (Bioplex PP, Alltech Inc., Nicholasville, KY, USA) at 1/3 levels of CON). Compared with the layers from CON group, the layers from ITM group had lower ($P < 0.05$) egg production, eggshell strength and yolk Fe concentration, and higher ($P < 0.05$) feed intake, egg loss and feed to egg ratio. The layers from OTM group had equivalent values for egg production, eggshell strength, egg loss and feed to egg ratio, but higher ($P < 0.05$) total egg weight and feed intake compared with those from CON group. The layers from OTM group had higher ($P < 0.05$) total egg weight, feed intake, egg production, eggshell strength, and yolk Fe concentration and lower ($P < 0.01$) egg loss and feed to egg ratio compared with those from ITM treatment group. The fecal samples from layers fed ITM diet and OTM diet had equivalent, but lower ($P < 0.01$) mineral (Fe, Cu, Mn and Zn) concentration compared with those from layers fed CON diet. The data from this study indicated that dietary supplementation of low-dose organic minerals as Bioplex PP can significantly reduce fecal excretion of trace elements and not negatively impact hen egg production and egg quality.

Key Words: organic trace minerals, production performance, eggshell quality, fecal mineral excretion, laying hen

502P Influence of iron source, level of supplementation and phytase level on broiler performance—Three-trial summary. J. Garrett*[1], G. Nunnery[2], and M. D. Sims[3], *[1]QualiTech Inc., Longmont, CO, [2]QualiTech Inc., Chaska, MN, [3]Virginia Diversified Research Inc., Harrisonburg, VA.*

Three trials were conducted to evaluate the influence of supplemental iron level, iron source and phytase level in the diets of broilers. This is a summary of those 3 trials. Trials lasted 42, 42, and 45 d in length. A total of 6800 birds (Ross 708) were fed. Dietary treatments evaluated were inorganic iron (iron sulfate; IFe) vs organic iron (SQM Fe, QualiTech, Inc.; OFe), level of supplementation 20 vs 60 ppm, phytase level of supplementation (0, 0.2, and 0.6 lb/ton; Quantum Blue – AB Vista; 0QB, 1xQB, and 3xQB). Stocking density was initially 0.6193 m^2/chick. Three phase diets were formulated starter, grower and finisher. Bird performance was evaluated at 21d and at the end of the study for weight gain, mortality and feed conversion value. Data was analyzed using GLM procedure in NCSS (Utah). There were no significant 2-way interactions between the treatments combinations, while there was a tendency ($P = 0.087$) for an interaction between phytase level and source of mineral. Level of iron supplementation had no significant effect ($P > 0.4$) on any performance parameter measured. Phytase level had a significant effect ($P < 0.001$) on bird weight gain for the first 21 d and

tended ($P = 0.054$) be greater at the end of the feeding period for the 6xQB. No treatment had a significant influence on bird mortality. The use of an organic source of iron had a significant effect ($P < 0.001$) on bird weight at 21 d and tended to have an effect on final bird weight ($P = 0.108$). There was a significant influence ($P < 0.05$) of organic iron source on feed conversion value (straight conversion and conversions adjusted for mortality and final body weight; 1.945 vs 1.857, 1.889 vs 1.813, 1.874 vs 1.816 for IFe and OFe, respectively) improving the feed conversion. Initial hypothesis was that source would allow for lower inclusion and possibly lower phytase levels but this was not confirmed. Instead, the organic iron source improved bird performance independent of the other treatments. This suggests that other unmeasured parameters were influence by iron source allowing for better bird performance, such as pathogen load, since certain pathogens (e.g., *E. coli*) have a requirement for iron which could create a subclinical infection rate. Further research is being pursued to evaluate this independent effect.

Key Words: broilers, organic iron, Inorganic iron, Quantum Blue phytase

503P Mineral source supplied during initial and production phases affects performance and egg quality of laying hens. H. Oliveira*[1], C. B. V. Rabello[1], M. Barros[1], M. do Carmo Ludke[1], A. Faria[1], W. Medeiros-Ventura[1], C. Pereira[1], A. Silva[1], R. S. Júnior[1], M. José dos Santos[1], L. Souza[1], L. H. Silva[1], and A. Fireman[2], *[1]Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brazil [2]Zinpro Corporation, Eden Prairie, MN.*

Studies have demonstrated the benefits of amino acid complexed minerals (AACM) on laying hen performance. Nevertheless, few studies have been completed that fed pullets from the starter period through the full production phase. An experiment was conducted to evaluate the effect of zinc (Zn), manganese (Mn), copper (Cu), and iron (Fe) amino acid complexes in laying hen diets, on their performance and egg quality. A total of 800 laying hens (Lohmann Brown Lite; 30 to 50 weeks of age) were distributed to cages (0.45 × 0.40 × 1.00 m) in a completely randomized design, with a 2 × 4 factorial arrangement of treatments. Each treatment had 10 replicate cages, with 10 birds per experimental unit (cage). Initial treatments were fed from day one to 30 weeks-of-age, and were either: Inorganic (IM), 70 ppm Zn + 70 ppm Mn + 8 ppm Cu as ZnO, MnO, and $CuSO_4H_2O$, respectively; or AACM-ZMC, 40 ppm Zn + 40 ppm Mn + 2.75 ppm Cu (from IM sources) and 30 ppm Zn + 30 ppm Mn + 5.25 ppm Cu (from AACM sources). During the production period, initial diet groups were divided into 8 treatments (4 groups per initial diet): IM; AACM-ZMC; AACM-ZMCFe (AACM-ZMC + 10 ppm Fe from $FeSO_4$ and 40 ppm Fe as Fe-AACM); AACM-Fe (IM + 10 ppm Fe from $FeSO_4$ and 40 ppm Fe-AACM). Data were submitted to ANOVA and Tukey's mean separation test ($P < 0.05$). Supplemental Zn, Mn, and Cu as AACM increased egg mass (EM) with or without Fe-AACM. Including Fe-AACM in the AACM-ZMC diet improved feed conversion ratio (FCR, g/g eggs), when compared with the Control (IM) sources. An interaction was observed between feeding phases and treatments for FCR (g/dz eggs); birds showed better FCR when fed AACM-ZMC, from initial through production phase. Birds fed diets containing solely IM in initial phase presented an inferior FCR in the production phase, even if fed AACM during the production phase. However, inclusion of Fe-AACM in production phase, with or without AACM-ZMC, improved this parameter. There were no interactions between treatments and feeding phases, related to egg quality parameters. Birds fed AACM diets had increased eggshell weight, eggshell thickness, and weight of egg and albumen, compared with those fed IM diets. Eggshell color was affected by mineral source, where diets containing AACM-ZMC, with or without Fe-AACM, and diets containing Fe-AACM, even without AACM-ZMC, promoted darker brown eggshells, compared with eggs from IM diets. Supplementing laying hen diets with AACM mineral during the initial and production phases improved performance and internal/external egg quality of layers.

Key Words: egg quality, eggshell quality, laying hen, mineral amino acid complex, mineral source

504P Effects of different sources and levels of copper and zinc on bone quality and mineral content in broilers. T. Santos*[1], K. Augusto[2], M. M. Sartori[1], P. Padilha[1], J. Vieira[1], R. F. Netto[1], D. Souza[1], L. Granero[1], M. Poletto[1], G. Barbosa[1], R. Araujo[1], and J. Sartori[1], *[1]São Paulo State University (UNESP), Botucatu, Brazil, [2]Trouw Nutrition, Campinas, Brazil.*

Micro minerals are extremely important for bone development, and ideal supplementation prevents the incidence of locomotor problems in broilers. Thus, the objective of the present study was to evaluate the effect of different sources (sulfate and hydroxychloride) and different levels of copper (Cu) and zinc (Zn) on bone quality (breaking strength and bone ash) in the tibia and femur and mineral content in tibia, femur and liver at 42 d-old.The latest sources of copper (Cu) and zinc (Zn) in broiler nutrition are hydroxychloride (IntellibondC; IBC and IntellibondZ; IBZ), which are less reactive and more stable in the diet and in gastrointestinal tract conditions when compared with inorganic sources. A total of 1,792 1-d old male Cobb chicks were distributed in a completely randomized factorial design in a 2x3+2: IBC (15 ppm and 150 ppm) × IBZ (80 ppm, 100 ppm and 120 ppm); Cu sulfate (15 ppm) × Zn sulfate (120 ppm); and Cu sulfate (150 ppm × Zn sulfate (120 ppm) with 8 treatments and 8 replicates of 28 birds each. The diets were formulated with corn and soybean meal, according to the recommendations of each phase: pre-starter (d1- to 7), starter (d8- to 21), growing (d22- to 35) and finishing (d36- to 42). At the end of the experiment, 2 birds/ replicate were slaughtered for collection of the liver, tibia and femur. The mineral content was determined by the technique of atomic absorption. Data were analyzed in a factorial 2x3+2 with the Minitab 16 statistical software, and the means were compared by the Tukey test ($P < 0.05$). The tibia breaking strength was higher ($P = 0.053$) for 150 ppm IBC × 80 ppm IBZ supplementation, while the level 150 ppm IBC × 120 ppm IBZ provided higher ash content in the tibia ($P = 0.027$). The copper content in the tibia ($P < 0.001$), femur ($P = 0.001$) and liver ($P = 0.008$) increased with 150 ppm IBC × 100 ppm IBZ, but the same treatment provided lower zinc content in the tibia. It was concluded that the supplementation of 150 ppm IBC was efficient for the parameters evaluated, whereas the IBZ supplementation showed variations with the levels used, therefore, 100 ppm IBZ can be chosen as an intermediate level. Acknowledgment: Trouw Nutrition, Fapesp (2017/00338-2), Capes (scholarship)

Key Words: bone resistance, bone ash, minerals, liver

505P Vitamin D_3 requirement for growing broiler chickens using a precision intubation intake bioassay. H. Leyva-Jimenez*, M. Khan, K. Gardner, R. Abdaljaleel, Y. AL-Jumaa, A. Alsadwi, and C. Bailey, *Texas A&M University System, College Station, TX.*

Vitamin D_3 (D_3) is obtained from the diet or is synthesized in vivo from the provitamin 7-dehydrocholesterol in the skin after exposure to UV-light. Current commercial facilities limit the access to sunlight. Therefore, D_3 supplements are used to prevent retarded growth and skeletal deformities. Classical D_3 requirement studies use graded levels

of dietary D_3 that are supplemented directly to the feed. Under this methodology, several sources of variation, including mixing error and selective feeding are frequent. The objective of this study focused on increasing the precision of D_3 delivery to the growing chick by performing a daily oral D_3 gavage. Using a completely randomized block design, commercial male d-old broilers were distributed in battery cages and fed a vitamin D_3-deficient corn-soy diet throughout a 21-d trial. The first 9 d of the study served to deplete the maternal stores of D_3 followed by a 12-h fasting period. From d-10 to the end of the trial, birds were orally gavaged with increasing levels of vitamin D_3. A highly purified pharmaceutical grade cholecalciferol standard (99.8%) was purchased from Sigma-Aldrich Chemical Company and dissolved in corn oil to create various D_3 treatments. Daily gavage treatments were based on estimated intake of 0, 50, 100, 200, 400, 800, 1,600 and 3,200 IU D_3/kg of feed consumed over the last 12 d of the study. The oral gavage was performed per treatment group from lower to higher IU concentration using an 18 gauge gavage needle and a 1 mL syringe graduated at 1/100 mL. Cholecalciferol intake per kg of diet was adjusted based on daily feed intake per pen of birds. Performance data were evaluated from d 10–21. Percent tibia bone ash (TBA), tibia breaking strength (TBS), total mineral content (BMC) and total bone mineral density (BMD) were obtained to evaluate bone mineralization at d 21. Data were subjected to ANOVA using the least squares function of JMP 13.0. Tukey's Test was used to identify significant ($P < 0.05$) differences when appropriate. Broken-line regression was used to calculate D_3 at the point of intersection between ascending line and horizontal line. Performance analysis resulted in no difference ($P > 0.05$) in body weight or weight gain. TBA and TBS were improved ($P < 0.05$) with increasing levels of D_3. No difference ($P > 0.05$) was found for BMD or BMC. Linear broken-line regression suggested a D_3 requirement of 491 IU/kg for TBA and 574 IU/kg for TBS. Whereas the quadratic broken-line regression suggested that 499 IU/kg and 799 IU/kg were required to maximize TBA and TBS respectively.

Key Words: cholecalciferol, vitamin D_3, requirement, broiler, bioassay

506P Evaluating vitamin premixes with various additives for vitamin and phytase stability in a commercial feed mill over six months. A. Levy* and N. Ward, *DSM Nutritional Products, Parsippany, NJ.*

Combining various additives into one premix allows for more flexible use of microbin space in feed mills. The objective of this study was to determine if inorganic minerals (oxides and sulfates), betaine, and NSP enzymes impact the stability of vitamins and phytase in a multicomponent vitamin premix over a 6-mo period in commercial feed mill conditions. Eight premixes were evaluated: 1) Vitamins + phytase (Control); 2) Control + NSP enzyme 1; 3) Control + betaine; 4) Control + Inorganic minerals (MIN); 5) Control + MIN + betaine; 6) Control + MIN + betaine + NSP enzyme 1; 7) Control + NSP enzyme 2; and 8) Control + MIN + betaine + NSP enzyme 2. The premixes were stored in a midwest US commercial feed mill for 6 mo under ambient conditions averaging 15°C (ranging from −18°C to 29°C). Samples were taken every 4 weeks from June through December 2016. The stability of vitamins A, D, E, thiamin, riboflavin, B6, and phytase in the premixes were evaluated. After 2 mo of storage, all premixes had less than 10% vitamin loss except premix 3, which had a 14% loss in riboflavin. Premix 3 had the lowest recovery of vitamin A, which was 72% of target after 6 mo. Of the vitamins analyzed, vitamin A suffered the highest losses with recoveries ranging from 72 to 89% of target across all premixes. Premixes 6 and 8 were the most complex, containing all additives for maximum microbin space savings. Premix 6 vitamin recovery was good, ranging from 91 to 103% except riboflavin was 75% at the end of 6 mo. Premix 8 vitamin recovery was also good, ranging from 86 to 129% except vitamin A was 76 and 80% of target at 5 and 6 mo, respectively. Phytase loss ranged from 0 to 20% with the greatest reduction in stability in premix 4, which contained only MIN with vitamins. With the exception of vitamin A, there were minimal losses of premix vitamins stored for 6 mo under commercial feed mill conditions.

Key Words: vitamin, premix, enzyme, phytase, stability

507P Effects of increasing manganese hydroxychloride level on male broiler growth performance and tibia strength. H. Williams*[1], A. Jasek[1], T. Parr[2], and J. Lee[1], *[1]Texas A&M University, Refugio, TX, [2]Micronutrients USA LLC, Indianapolis, IN.*

The objective of the current study was to evaluate the impact of increasing manganese (Mn) hydroxychloride on growth performance and tibia strength of male broilers fed corn-soy diets. A total of 2,100 broilers were assigned randomly to 5 dietary treatments consisting of 12 replicates of 35 Ross 708 broilers per pen. Dietary treatments contained manganese from Mn hydroxylchloride (IntelliBond M; Micronutrients USA LLC) at 0, 40, 80, 120, and 160 ppm respectively. Broilers were fed starter (d1–14), grower (d14–28), finisher (d28–42), withdrawal 1 (d42–49), and withdrawal 2 (d49–55) diets. Average body weight (BW), body weight gain (BWG), mortality adjusted feed conversion ratio (FCR), feed consumption (FC) and mortality (%) were determined on d 14, 28, 42, 49, and 55. On d 42 and 55, 5 birds per replicate pen were randomly selected and right tibias were excised for evaluation of bone breaking strength and inorganic matter. All data were analyzed via a one-way ANOVA with means considered significantly different a $P < 0.05$ and were further separated using Duncan's multiple range test. Regression analyses for FCR were analyzed with increasing levels of Mn. No differences in body weight were observed among Mn level through d 49. On d 55, broilers fed 80 and 160 ppm Mn were heavier ($P < 0.05$) than those fed 40 ppm. Through d 42, 49, and at termination of the study, broilers fed diets containing elevated levels of Mn at 80, 120, and 160 ppm had improved ($P < 0.05$) FCR compared with the birds fed diets containing 0 ppm. The regression of FCR on dietary Mn had a negative slope which was significantly different from zero indicating positive impacts on FCR with increasing levels of Mn ($P < 0.05$) through d 14, 28, 42, 49 and 55. At the termination of the study, broilers fed Mn at 160 ppm exhibited improved ($P < 0.05$) tibia breaking strength as compared with broilers fed 80 and 120 ppm Mn. These data indicate that the dietary concentration of Mn needed to maximize growth performance is higher than the reported value in the NRC (1994) of 60 ppm. Additionally, the requirement to maximize bone strength is higher than the requirement to maximize body weight.

Key Words: manganese, broiler, performance, ash

508P Effect of dietary addition of iron sulfate in broilers diet on water-soluble phosphorus excreted, minerals digestibility and bone mineralization. P. Floradin*, F. Guay, and M.-P. Létourneau-Montminy, *Laval University, Quebec, QC, Canada.*

Soluble P is highly bioavailable to macrophytes and algae and therefore readily affects water quality. Phosphates are commonly removed from municipal and industrial wastewater by precipitation with multivalent metals such as iron (Fe). In a previous trial it has been shown that the addition of iron sulfate encapsulated can reduced the excretion of water-soluble P (WSP) in excreta without modifying P retention and growth performance. The impact Fe encapsulated added in broiler diet on WSP

excretion in litter was the subject of the present trial. In addition, the effect of iron encapsulated or not using a spray-chilling method was also tested in terms of growth performance, apparent jejunal and ileal (AID) digestibility of calcium (Ca), Fe, P and phytic P (PP), kidneys and liver Fe content, and bone mineral content (BMC) of tibia (DXA, Discovery W; Hologic Inc., Waltham, MA, USA). The experiment was divided in 3-phases feeding program (0–10, 10–21, 21–34 d) using 5 diets that meet all nutritional requirements except Fe: a positive control (C+); C+ supplemented with 33 and 60 ppm Fe sulfate encapsulated (FeE1 and FeE2), and C+ supplemented with 33 and 60 ppm Fe sulfate non-encapsulated (FeNE1 and FeNE2). Two thousand 7 hundred (2700) 1 d-old Cobb-500 male broilers were assigned to 1 of 5 diets and distributed in 60 pens of 45 birds each. Six birds per pen were euthanized by cervical dislocation at d 30 and 31. Data were analyzed as a randomized complete block design with PROC MIXED of SAS to test the impact of 1) Fe addition (C+ vs other), 2) Fe Encapsulation (FeE1-FeE2 vs FeNE1-FeNE2), 3) Dose effect of FeE (FeE1 vs FeE2), and 4) Dose effect of FeNE (FeNE1 vs FeNE2). It should be noted first that iron in the C+ diet was higher than expected (~300 ppm). Overall ADG was reduced (C+ vs other, $P = 0.05$) by iron addition except in birds receiving FeE1 (FeE1 vs FeE2, $P < 0.05$). Similarly, BMC was reduced with iron addition (C+ vs other, $P = 0.02$) except in FeE1 (FeE1 vs FeE2, $P = 0.009$). A significant increase in AID of total P ($P < 0.001$), PP ($P = 0.01$), Ca ($P < 0.001$) and Fe ($P < 0.001$) was observed with the addition of Fe (C+ vs other). These effects were stronger with FeNE (FeE vs FeNE) for total P ($P < 0.001$) and PP ($P = 0.01$). Iron did not significantly affect WSP concentration in the jejunum, ileum, but increase WSP in the litter (C+ vs other, $P < 0.001$). These results suggest that Fe addition reduces growth performance and does not insolubilize the P in the litter. This is partly due to the adverse effect of high Fe concentration on bone mineralization and remodeling, inducing an increase in urinary excretion of P. The increase of P digestibility with iron addition needs further investigation.

Key Words: water soluble-phosphorus, iron sulfate, apparent ileal digestibility, broiler

509P Impacts of increasing level of a novel organic zinc complex on performance and bone mineral content in young male broilers. D. De Leon*[1], K. Brown[1], F. Munoz[2], R. Poureslami[3], and J. Lee[1], *[1]Texas A&M University, College Station, TX, [2]Scott G. Williams LLC, Conyers, GA, [3]ADM Animal Nutrition, Dallas, TX.*

An experiment was conducted to determine the bioavailability of a novel zinc complex using tibia zinc concentration as the response criteria. The effect of the complex on growth and bone deposition was determined in Cobb 500 broiler chicks which were fed a semi-purified diet containing soy protein concentrate, dextrose, and corn starch (18 mg/kg of background Zn). Twelve replicate pens of 10 birds were permitted access to experiment diets from d 1 through 21 d of age in a battery trial. The control diet was supplemented with zinc complex at 10, 20, and 40 mg/kg. Parameters evaluated were feed consumption, body weight, mortality corrected feed conversion ratio and tibia zinc concentration. The data were analyzed via one way ANOVA using the GLM procedures and linear and quadratic regression was conducted to determine slope of evaluated parameters. Body weight was increased with all levels of zinc complex inclusion compared with the control diet which was related to an increase in feed consumption. No consistent dose-dependent improvements were observed in the mortality corrected feed conversion ratio. Using zinc deposition in bone as the response measurement, consistent dose-dependent increases were observed in tibia zinc concentration, as significant increases in tibia zinc concentration was observed with each increase in zinc complex inclusion. Linear and quadratic regression confirms increasing levels of zinc complex resulting elevated BW and tibia zinc content. These data confirms the bioavailability of this novel zinc complex to broiler chickens.

Key Words: zinc complex, tibia, broiler

510P Interactive effects of probiotics and copper inclusion on 42 d male broiler performance. A. Brown*[1], M. Lemons[1], K. Peryman[2], A. Kiess[1], and K. Wamsley[1], *[1]Mississippi State University, Mississippi State, MS, [2]Micronutrients USA LLC, Indianapolis, N.*

The recent rise in antibiotic-free production has led nutritionists to rely on feed additives such as probiotics and copper as potential antibiotic alternatives. Probiotics, such as those derived from *Bacillus licheniformis* (BL) have been found to alter the environment of the GIT, activate the immune system and promote colonization of beneficial microorganisms, ultimately improving overall performance. Copper, such as that supplied via Cu hydroxychloride (Micronutrients USA, LLC) (CU), is a micro-mineral with various roles in vivo, including regulating immune function; thereby promoting its use for antibiotic-free production. Research has explored their individual use (BL and CU) in diets, however research is limited on their combined effect on broiler performance. Therefore, the objective of the current study was to examine the individual/interactive effects of supplementing commercially available BL (Yes or No) or CU (0, 125, or 250 ppm) in an all-vegetable commercial diet on d 0–42 male broiler performance. This study used a 2 BL × 3 CU factorial arrangement of treatments within a RCBD. A total of 1,152 Ross × Ross 708 male broilers were obtained from a commercial hatchery and equally allocated to 96 floor pens containing used litter (16 replications/treatment). Birds were weighed and feed intake was recorded on d 18, 30 and 42 for determination of average BW, BW gain, bird uniformity, feed intake/bird (FI), % mortality, and feed conversion ratio (FCR). No differences were found for BL, CU or their combination at 0–18 or 42 d ($P > 0.05$). However, BL × CU interactions were established for 18–30 d BW gain ($P = 0.01$) and 0–30 d FI ($P = 0.04$). For 18–30 d, including BL+CU into diets decreased BW gain; however, for 0–30 d FI, CU alone increased FI while including BL+ CU decreased FI. On 0–30 d, regardless of FI, FCR was not found to be significantly affected ($P > 0.05$). Copper inclusion was found to significantly affect d 30–42 BW gain with broilers receiving 250 ppm CU having the highest BW, while broilers fed all other CU inclusions performed similarly ($P = 0.02$). On d 42, 5 birds/treatment were randomly selected for cecal tonsil sampling to determine the presence or absence of *Salmonella* or *E. coli*. While *Salmonella* was not detected, birds fed diets containing CU had less *E. coli* as compared with birds fed diets without CU ($P = 0.04$); BL had no effect on *E. coli* ($P > 0.05$). Data from this study were more positive for CU inclusion and indicated some concern for the combination of CU and BL. It is possible that replicating this study under higher disease challenge could elicit different result, as overall flock health for this study was good (regardless of the use of used litter).

Key Words: copper, antibiotic-free, antibiotic alternative, probiotic, broiler

511P Effects of different selenium sources on egg quality of semi-heavy laying hens. F. P. Costa*[1], A. Ferreira de Brito[1], M. N. Soares[1], T. S. Ferreira[1], J. V. Celestino da Silva[1], D. T. Cavalcante[2], J. C. L. Muniz[1], J. G. Gonçalves[3], and M. Ceccantini[3], *[1]Federal University of Paraiba, Areia, Paraiba, Brazil, [2]Academic Unit of Garanhuns, Garanhuns, Pernambuco, Brazil, [3]Adisseo South America, Sao Paulo, Brazil.*

Selenium is a essential mineral that is involved in several biochemical processes and is fundamental to the formation and production of eggs, exerting influence on their quality. This trial was carried out to evaluate the effects of different selenium sources on laying hen egg quality traits from 50 to 70 weeks of age. A total of 384 Dekalb Brown laying hens (average body weight of 1.86 kg) were randomly assigned to one of 2 dietary treatments with 12 replicates of 16 hens. Experimental treatments consisted of the same dietary level of Se (0.3 ppm) supplied by sodium selenite or hydroxy-selenomethionine (Selisseo). Both diets were isonutritive, being formulated to meet or exceed the nutrient recommendations described by the genetic strain guideline. Egg quality traits analyzed included weight and percentage of albumen (g, %), yolk (g, %), and eggshell (g, %), eggshell thickness (mm) and strength (kg/f), specific egg weight (g/cm3), Haugh unit, and yolk color. Data were analyzed as one-way ANOVA and means were compared using T student comparison test, considering statistical differences for $P < 0.05$. Selisseo increased ($P < 0.05$) egg weight (63.63 vs. 62.50g), albumen weight (40.37 vs. 39.40g), eggshell thickness (0.470 vs. 0.458mm), eggshell strength (3.335 vs. 3.092kgf) compared with sodium selenite. However, the other egg quality parameters were not influenced ($P > 0.05$) by selenium sources evaluated. The supplemented of 0.3 ppm of hydroxy-selenomethionine (Selisseo) improves eggshell quality traits of laying hens from 50 to 70 weeks of age compared with the traditional inorganic source sodium selenite.

Key Words: hydroxy-selenomethionine, eggshell strength, eggshell thickness, albumen

512P Effect of Selenium supplementation on performance of laying hens. F. P. Costa*[1], A. Ferreira de Brito[1], T. S. Ferreira[1], J. V. Celestino da Silva[1], D. T. Cavalcante[2], J. C. L. Muniz[2], J. G. Gonçalves[3], and M. Ceccantini[3], *[1]Federal University of Paraiba, Areia, Paraiba, Brazil, [2]Academic Unit of Garanhuns, Garanhuns, Pernambuco, Brazil, [3]Adisseo South America, Sao Paulo, Brazil.*

The supplementation of diets with Selenium is made to meet the nutritional requirements of poultry, with positive effects on performance. However, few studies about sources and levels of selenium for laying hens have been done and the results are contradictory. This experiment was carried out to evaluate the effects of different selenium sources on the performance of laying hen from 50 to 70 weeks of age. A total of 384 Dekalb Brown laying hens (average body weight of 1.86 kg) were randomly assigned to one of 2 dietary treatments with 12 replicates of 16 hens. Experimental treatments consisted of the same dietary level of Se (0.3 ppm) supplied by sodium selenite or hydroxy-selenomethionine (Selisseo). Both diets were isonutritive, being formulated to meet or exceed the nutrient recommendations described by the genetic strain guideline. Parameters analyzed were feed intake (FI, g/d), egg production (%), egg weight (g/egg), egg mass (g/d), egg mass conversion (g/g), egg dozen conversion (kg feed/dozen eggs), number of eggs per poultry housed (egg/poultry housed), viability (%) and final weight of hens (g/bird). Data were analyzed as one-way ANOVA and means were compared using T student comparison test, considering statistical differences for $P < 0.05$. It was observed that the use of Selisseo resulted in an increase ($P < 0.04$) in egg production and number of eggs per poultry housed, respectively, of 1.79% and 3.63%, relative to sodium selenite. In relation to egg weight and egg mass, it was verified that sodium selenite supplementation provided lower ($P < 0.02$) egg weight (63.63 vs. 62.50g) and egg mass (57.54 vs. 55.50 g) when compared with Selisseo supplementation. These results influenced the conversion values by mass and dozen eggs, which were lower ($P < 0.05$) with the use of Selisseo in the diets (1.945 kg / kg and 1.484 kg / dozen), compared with selenite sodium (2.053kg / kg and 1.540kg / dozen). The other variables of productive performance were not influenced ($P > 0.05$) by selenium sources evaluated. The supplementation of 0.3 ppm selenium from Selisseo improves the performance of laying hens aged between 50 and 70 weeks of age.

Key Words: hydroxy-selenomethionine, organic trace mineral, egg production

513P Recovery of *Salmonella* and *Campylobacter* in various concentrations of cetylpyridinium chloride. D. Cosby*, G. Gamble, N. Cox, S. Mize, M. Berrang, and A. Hinton, *USDA, ARS, USNPRC, Athens, GA.*

Use of biocides in US poultry processing plants has become a matter of concern with regards to recovery of human enteropathogens from carcass rinses collected for regulatory analysis. Cetylpryridinium chloride (CPC) is one of the most prevalent biocides being used in the processing plant to reduce *Salmonella* and *Campylobacter*. This study evaluated the recovery of *Salmonella* and *Campylobacter* species from overnight storage in 1:2 serial dilutions of CPC (1000 to 0.49 ppm) at 4°C. Eleven *Campylobacter* cultures (5 – *C. jejuni* and 6 – *C. coli*) commonly isolated from poultry were grown on Campylobacter-CEFEX (CEFEX) agar plates at 42°C for 48 h under microaerobic conditions and 10 *Salmonella* serotypes (commonly isolated from poultry) were grown on brilliant green sulfa (BGS) plates overnight at 37°C before each replication. Serial dilutions of CPC and propylene glycol (PG) were prepared in plastic 24-well tissue culture plates containing buffered peptone water (BPW). An average of 10^7 colony forming units (cfu) of each enteropathogen was added to each well. Inoculated plates were stored in plastic zip lock bags overnight at 4°C. After overnight storage, 10 µL of the stored broth was streaked for isolation onto CEFEX agar plates for *Campylobacter* and onto BGS agar plates for *Salmonella.* CEFEX plates were incubated at 42°C for 48 h under microaerobic conditions and BGS plates were incubated at 37°C for 24 h, the cfu were enumerated. Five replications were conducted on separate days. *Salmonella* serotypes chosen for use in this study were susceptible to very low concentrations of CPC; growth was detected only at 2 ppm or lower. *Campylobacter* strains were similar in susceptibility with growth recorded at 7.8 ppm or lower. These data indicate that residual CPC which may remain attached to the carcass after processing may adversely affect recovery of *Salmonella* and *Campylobacter* species from carcass rinses used by regulatory agencies to determine the safety of processed poultry products and may result in negative observations and provide a false sense of security to consumers.

Key Words: *Salmonella*, *Campylobacter*, in vitro, cetylpyridinium chloride

514P Antibiotic profiles of bacteria isolated on selective *Campylobacter* media. A. Hinton*, M. Rothrock, D. Cosby, K. Ingram, and N. Cox, *USNPRC, ARS, USDA, Athens, GA.*

The objective of this study was to determine the antibiotic résistance (AR) profiles of non-*Campylobacter* bacteria recovered on selective *Campylobacter* media. Broiler carcasses were obtained from a local commercial poultry processing facility, and whole-carcass rinses were performed by shaking carcasses in plastic bags with 200 mL of distilled water for 2 min. Rinsates were decanted, and serial dilutions of the rinsates were prepared. Selective *Campylobacter* agar supplemented with Blaser-Wang antibiotic mixture and selective *Campylobacter* aerobic (CAE) broth supplemented with Bolton's antibiotic mixture were inoculated with serial dilutions of the rinsates. The selective agar plates were incubated microaerobically in GasPak jars, and CAE was incubated aerobically for 48 h at 37°C. After CAE incubation, serial dilutions of the media were also spread-plated on selective *Campylobacter* agar and incubated microaerobically for 48 h at 37°C to isolate bacteria that could grow on both media. Morphologically distinct olonies were selected from both sets of agar plates and identified using the Biolog Bacterial Identification System. Antimicrobial susceptibility of the isolates were determined using Sensititer Antimicrobial Susceptibility Testing Plates containing the antibiotic series and concentrations of the CDC National Antimicrobial Resistance Monitoring System for enteric bacteria. Data was analyzed determine the AR resistance of each isolate to construct AR profiles. Isolates recovered from direct plating of rinsates onto selective agar included *Acinetobacter* spp., *Ochrobactrum* spp., *Pseudomonas* spp., *Rhizobium radiobacter*, and *Stenotrophomonas maltophilia*. *Ochrobactrum* spp. and *Pseudomonas fulva* were recovered from selective CAE plated on selective agar. All isolates were susceptible to azithromycin, ciprofloxacin, ceftriaxone, and gentamicin; and resistant to cefoxitin. Only *Ochrobactrum anthropi* was resistant to streptomycin and only *R. radiobacter* was resistant to tetracycline. Pseudomonads were resistant to nalidixic acid and sulfisoxazole, while pseudomonads and *S. maltophilia* were resistant to ampicillin. Pseudomonads and *Acinetobacter* spp. showed the highest resistance to chloramphenicol. Additionally pseudomonads, *O. anthropi*, and *S. maltophilia* were resistant to amoxicillin-clavulanate; while these isolates and *A. guillouiae* showed resistance to ampicillin. Conclusions indicated that each isolate possessed a distinctive AR profile. These findings may be used to formulate improved supplements to reduce growth of contaminants on selective *Campylobacter* media.

Key Words: *Campylobacter*, poultry, selective media, Blaser-Wang antibiotics, Bolton's antibiotics

515P Validation of applying electrostatic versus conventional sprayer with commercial antimicrobials to inactivate *Salmonella*, *Listeria monocytogenes*, and *Campylobacter* on eggs. W. Jiang*, K.W. Li, L. Lemonakis, and C. Shen, *West Virginia University, Morgantown, WV.*

The cross-contamination in the hatchery from foodborne pathogen infected eggs to baby chicks has been considered as a critical issue in poultry industry. Electrostatic spraying is an emerging technique that can improve the distribution and coverage of antimicrobials during egg sanitation. This study aims to compare the efficacy of commercial antimicrobials sprayed by electrostatic sprayer versus conventional sprayer to inactivate *Salmonella*, *Listeria monocytogenes* and *Campylobacter jejuni* on egg surfaces. Experiments were conducted by 2 X 5 factoral design with electrostatic sprayer versus conventional sprayer (2 factors) and 5 different antimicrobials for *Salmonella*, *L. monocytogenes* and *Campylobacter jejuni.* Eggs were dip-inoculated with overnight cultured (18-h) *Salmonella* Typhimurium and Tennessee, 2-strain mixture of *L. monocytogenes*, and 2-strain mixture of *C. jejuni* (microaerophilic condition) followed by unsprayed, electrostatic spraying (Electrostatic Spraying Systems BP2) and conventional spraying (Chapin 1-Gallon Plastic Tank Sprayer) peroxyacetic acid (PAA, 0.1%), lactic acid (LA, 5.0%), lactic and citric acid blend (LCA, 2.5%), sodium hypochlorite (chlorine, 50 ppm), and SaniDate-5.0 (SD, a mixture of PAA and H_2O_2, 0.25%) to the surface of each egg for 30 s (15s each side). Eggshells were aseptically removed and placed into 50 mL of buffered peptone water followed by shaking and massaging for 1 min. Survival bacteria were recovered onto XLT-4 (*Salmonella*), Modified Oxford agar (*L. monocytogenes*), Brucella agar (*C. jejuni*). Data (2 repeats/6 samples/repeat) were analyzed using the Mixed Model Procedure of JMP and confirmed with SAS. Initial population of *Salmonella*, *L. monocytogenes* and *C. jejuni* recovered on eggs were 5.52–5.64, 5.81–6.40,

and 4.24–4.81 log cfu/egg. Overall, electrostatic sprays of all 5 tested antimicrobials were more effective ($P < 0.05$) than conventional sprays in decontaminating *Salmonella* (LSMeans 2.24 vs 0.88 log cfu/egg), *L. monocytogenes* (LSMeans 2.53 vs 1.11 log cfu/egg) and *C. jejuni* (LSMeans 1.61 vs 0.66 log cfu/egg) on eggs. Compared with the conventional sprayer, electrostatic spraying PAA, SaniDate, and chlorine achieved additional ($P < 0.05$) reductions of 0.96–3.18, 1.19–3.05, 0.96–1.62 logCFU/egg for *Salmonella*, *L. monocytogenes*, and *C. jejuni* respectively. The results indicated that electrostatic sprayer with commercial antimicrobials can be considered as an effective approach to enhance microbial safety of eggs.

Key Words: *Salmonella*, *Listeria*, electrostatic spray, egg, *Campylobacter*

516P 16S rDNA-based sequencing as a rapid method to identify bacteria cultivated from nonselective aerobic plate bacterial colonies isolated from commercial poultry feed. A. Micciche*, L. Meyer, and S. Ricke, *University of Arkansas, Fayetteville, AR.*

The microbial composition of poultry feed could be a critical factor for the development and growth of broilers and their gut microbiome. Poultry feed harbors a wide-array of microorganisms, some of which are pathogens. However, little is known about the taxonomy of non-pathogenic bacteria associated with commercial feeds. This is important since the presence of pathogens are relatively infrequent; therefore, gaining a better understanding of non-pathogens may aid in the identification of more representative indicator organisms. These novel indicator organisms could in turn be used to assess the potential for the presence of pathogens. This would have utility for quantitating the effect of feed additives on these candidate organisms. However, conventional means for identifying and characterizing feed microbial isolates requires selective enrichment and cultivation, is slow, and can result in loss of viable non-culturable bacteria. Instead, isolating bacteria from non-selective media that supports a more diverse viable microbial population, followed by sequencing the 16S rDNA gene of these isolates, offers a much more rapid means to not only screen poultry feeds but precisely identify individual organisms. The objective of this study was to identify typical aerobic isolates from commercial poultry corn-based feed and assign them their corresponding taxonomy. Ten grams of corn feed was added to 100 mL of tryptic soy broth in a sterile stomacher bag and then manually shaken for 2 min. Serial dilutions were performed up to 10^{-3} in PBS and plated onto tryptic soy agar (TSA) and aerobic plate count agar (APC). Plates were incubated at 25°C for 2 d. Twenty distinct colonies were selected and isolated on TSA. DNA was extracted from 3 independent replicates using the gram-positive protocol for the DNeasy Blood and Tissue kit. DNA was sequenced using an Illumina MiSeq sequencer. Eleven isolates were successfully purified, sequenced, and annotated and taxonomy assigned by the MOTHUR pipeline. Colonies that were identified by this approach included *Enterococcus* spp., *Pantoea* spp., *Stenotrophomonas* spp., and *Enterobacteriaceae* spp. Interestingly, some colonies isolated proved to be actually a mixture of organisms such as *Campylobacter*, *Enterobacteriaceae*, and other gram-negative bacteria. The results of this study indicate that feed microbial populations recovered on aerobic plates is variable. These results confirm that 16S rDNA sequencing is a viable method for rapidly identifying microorganisms recovered from non-selective media.

Key Words: 16S sequencing, non-selective media, poultry feed, rapid identification

517P Evaluate the efficacy of applying a portable electrostatic sprayer with commercial antimicrobials against unstressed and starvation-stress-adapted *Campylobacter jejuni* on broiler wings. L. Lemonakis, W. Jiang*, K. W. Li, and C. Shen, *West Virginia University, Morgantown, WV.*

Our recent study showed that 40% of broilers processed at a West Virginia small USDA-inspected facility were positive for *Campylobacter*. Electrostatic spraying is an emerging technique that can improve the distribution and coverage of antimicrobials during decontamination of foodborne pathogens on poultry meat products. This study aims to evaluate the inactivation of unstressed and starvation-stress-adapted *Campylobacter jejuni* on broiler wings sprayed by commercial antimicrobials using a portable electrostatic sprayer. Experiments were conducted by 2 × 5 factorial design with unstressed versus starvation-stress-adapted cells (2 factors) and 5 different antimicrobials (5 factors). Overnight cultured (18-h) 3 *C. jejuni* strains were unstressed, or sub-cultured in 0.9% saline solution for 2-h to prepare starvation-stress-adapted cells. Fresh thawed broiler wings were spread-inoculated with prepared *C. jejuni* solutions, followed by unsprayed, and electrostatic sprayed (Electrostatic Spraying Systems BP2) with peroxyacetic acid (0.1%), lactic acid (5.0%), lactic and citric acid blend (2.5%), sodium hypochlorite (chlorine, 50 ppm), and SaniDate-5.0 (a mixture of PAA and H_2O_2, 0.25%) to the surface for 30 s (15 s each side). Surviving bacteria were recovered onto Brucella agar under microaerophilic condition. Data (2-repeats/3–4 samples/repeat) were analyzed using the Mixed Model of SAS. Initial population of unstressed and starvation-stress-adapted *C. jejuni* recovered on wings were 4.68 and 4.64 $\log_{10}$cfu/ml. Unstressed and starvation-stress-adapted cells behaved differently to antimicrobials with a significant effect of antimicrobials ($P < 0.05$), stress ($P < 0.05$), and the interaction ($P < 0.05$). Electrostatic spraying antimicrobials reduced ($P < 0.05$) the unstressed *C. jejuni* by 2.21–2.54 $\log_{10}$ cfu/ml compared with the control. Compared with the unstressed cells, reductions of starvation-stress–adapted cells indicated cross-protection (0.83–1.03 $\log_{10}$ cfu/ml) of *C. jejuni* during subsequent exposure to antimicrobials. However, there is no difference ($P > 0.05$) of reduction among 5 tested antimicrobials using the portable electrostatic sprayer regardless of unstressed or starvation-stress-adapted cells. Applying post-chilling antimicrobial treatments in a portable electrostatic sprayer could effectively reduce *Campylobacter* on broiler parts. Future challenge studies of broiler meat should include stress-adapted-cells since cross-protection could be generated by starvation-stressed cells.

Key Words: *Campylobacter*, stress, electrostatic spray, broiler, wing

518P DNA sequences and protein structures of target genes *prot*6E and *sef*A for detecting *Salmonella* Enteritidis. L. Hu*[1], R. Stones[2], E. Brown[1], M. Allard[1], L. M. Ma[3], and G. Zhang[1], [1]*FDA, College Park, MD,* [2]*Newcastle University, Newcastle upon Tyne, United Kingdom,* [3]*Oklahoma State University, Stillwater, OK.*

Genes *prot*6E and *sef*A are frequently used as targets for the detection of *Salmonella enterica* serotype Enteritidis (*S.* Enteritidis). We attempted to investigate the variation of these genes among *S.* Enteritidis strains and predict the protein structures of the genes using WGS data. WGS of 68 *S.* Enteritidis isolates from egg and chicken samples were conducted using Illumina technologies, and the isolates were identified with 3 distinct clades based on the generated phylogenetic tree. *prot*6E and *sef*A genes were observed in all 68 isolates studied. Comparative genomic analysis indicated 2 noticeable non-synonymous mutations (Glycine à Serine and Valine à Isoleucine) of *prot*6E in 11 *S.* Enteritidis isolates (9 egg samples, 2 chicken samples) among the 68 isolates. However, the protein structure of these 2 mutations could not be well modeled, using SWISS-MODEL. There was one significant non-synonymous mutation (Valine à Glutamic Acid) occurred in *sef*A gene in 4 *S.* Enter-

itidis isolates from egg samples of the 68 isolates. Protein structure of the mutant was modeled successfully, which was clearly different from that of the regular isolates. The circular maps of plasmid genomes from 2 PacBio platform-sequenced *S.* Enteritidis isolates illustrated *prot6*E gene located on the tail of the plasmid. Based on the Biosynthesis of amino acids - Reference pathway in the KEGG pathway Database, the transition of amino acid of *sef*A Var. was a transversion from essential amino acid to non-essential amino acid, while that of *prot*6E Var.1 happened between the conditionally non-essential amino acids, and *prot*6E Var. Two occurred between essential amino acids. On the other hand, the variety properties of the mutated amino acids, like the side-chain polarity and charge, might contribute to the occurrence and rate of mutation in *prot*6E and *sef*A genes. These results provide better understanding of *S.* Enteritidis in general, *prot*6E and *sef*A genes in specific, and will help design better detection methods for *S.* Enteritidis.

Key Words: *Salmonella* Enteritidis, prot6E, sefA

519P Whole-genome sequencing and virulence characterization of *Campylobacter jejuni* strains isolated from poultry. A. Upadhyay*[1], I. Upadhyaya[1], S. Shrestha[1], K. Arsi[1], S. Shrestha[1], A. Donoghue[2], and D. Donoghue[1], *[1]University of Arkansas, Fayetteville, AR, [2]Poultry Production and Product Safety Research Unit, ARS, USDA, Fayetteville, AR.*

Campylobacter jejuni is a major foodborne pathogen that causes severe gastroenteritis in humans. Chickens act as the host for *C. jejuni*, wherein the pathogen colonizes the ceca thereby leading to contamination of the carcass during slaughter and subsequent human infections. In the human gut, the pathogen attaches and invades the intestinal epithelium followed by toxin mediated cytopathy and enteritis. Little is known about how this pathogen is able to colonize multiple hosts while competing with specialist microbiota in the gut. Comprehensive characterization of *C. jejuni* strains could facilitate in better understanding of their pathophysiology and development of effective intervention strategies. Whole genome sequencing is a powerful technology that provides in-depth genetic information and is increasingly being utilized to study the evolution, epidemiology, virulence, and detection of foodborne pathogens. Herein, we report the complete genomic sequence of 4 wild-type *C. jejuni* strains (called, S1, S3, S4 and S8), isolated from poultry in the United States. In addition, mechanistic analysis was conducted to test the colonization potential and virulence attributes of the strains. Whole genome sequencing, de novo assembly and genome annotation revealed a chromosome of 1,714,057, 1,671,321, 1,685,319 and 1,690,693 bp in isolates S1, S3, S4 and S8, respectively. The genome GC content of all strains was between 30.9 and 31.2% with 1718 to 1861 coding sequences and 44–49 RNAs. Multiple genes coding for virulence factors such as motility, toxin production, and stress tolerance were observed in all tested strains. In addition, genes imparting resistance to antibiotics such as β-lactams and toxic compounds such as copper and arsenic were also found in all 4 strains. The *C. jejuni* genome of tested strains also revealed CRISPR sequences imparting adaptive immunity against phage invasion. Follow up mechanistic analysis showed that all strains were capable of motility at 42°C and 37°C, biofilm formation and attachment to epithelial cells from chickens and humans. Taken together, the genomic data from these potentially virulent strains should provide a better understanding of the colonization and pathogenesis mechanisms of *C. jejuni* leading to better control strategies in poultry. Comparative analysis outside the conserved core genomes of these 4 strains is currently underway.

Key Words: whole-genome sequencing, *Campylobacter jejuni*, virulence, poultry, safety

520P A meta-analysis on the use of probiotics to reduce *Salmonella* in broilers. A. Micciche*[1], C. Rainwater[1], J.A. Lee-Bartlett[1], K. Feye[1], D. McIntyre[2], H. Pavilidis[2], and S. Ricke[1], *[1]University of Arkansas, Fayetteville, AR, [2]Diamond V, Cedar Rapids, IA.*

Numerous studies have evaluated the effects of probiotics in poultry to reduce *Salmonella*. However, significant variability exists between the conclusions in executed, peer-reviewed research. Therefore, there is a significant need to quantitatively evaluate the literature and determine the efficacy of yeast and bacteria probiotics. A meta-analysis was employed to evaluate probiotic efficacy in reducing *Salmonella* in broilers. Inquiries evaluated the impact of mixed versus single microbial strain probiotics against *Salmonella* cecal colonization. Eight databases were selected with the assistance of the University of Arkansas library system, using the search phrase "probiotic" AND "*Salmonella*" AND "chicken," with the term "reduce" added for one database search. The search results were pooled with EndNote Basic. The exclusionary criteria included: papers had to be written in English, peer-reviewed, fit search criterion, and reported *Salmonella* in ceca. Of the 3,243 reviewed papers, 8 were suitable for inclusion. Two trained reviewers independently reviewed the studies and extracted appropriate data. Data was imported into Graphpad and analyzed via Welch's *t*-test for multiple comparisons ($P < 0.05$). Four different axenic cultures of probiotics, *Saccharomyces cerevisiae, Lactobacillus plantarum*, *L. johnsoni*, and *Enterococcus faecium* were used. Commercial probiotics were also evaluated, as were synbiotics. The most common method for delivery was oral gavage, which served as the most common means of exposure ($P < 0.0001$). Finally, mixed probiotic cultures were 1.5 times more commonly used than axenic cultures. Despite the diversity in research design and probiotic selection, several probiotics appeared more effective against *Salmonella*, with a total reduction of *Salmonella* shedding by 39% across all studies ($P < 0.001$) assessed in this analysis. These results may be used to more efficiently select optimal on-farm interventions anti-*Salmonella* control measures in broilers.

Key Words: meta-analysis, *Salmonella*, probiotics, broiler, ceca

521P Effect of sodium bisulfate salt on reducing the presence of an antibiotic resistant *Salmonella* Typhimurium on whole chicken wing parts. J. Atchley*[1], D. Dittoe[1], K. Feye[1], L. Meyer[1], C. Knueven[2], and S. Ricke[1], *[1]University of Arkansas, Faytteville, AR, [2]Jones-Hamilton Co., Walbridge, OH.*

The contamination of processed poultry products by *Salmonella* spp. is a major concern in the poultry industry as salmonellosis is one of the leading causes of food borne illness in the United States. For this reason, new antimicrobial solutions are being tested for their efficacy in reducing such pathogens in industry settings. The objective of this study was to determine the efficacy of concentrations of Sodium Bisulfate Salt (SBS), alone and in combination with peracetic acid (PAA), in reducing populations of nalidixic acid resistant (NA) *Salmonella* Typhimurium on whole chicken wing parts. A total of 24 (8 treatments, 3 d) whole wing parts (WP) were inoculated with 10^7 cfu/mL of nalidixic acid (NA) resistant *S.* Typhimurium in 400mL of sterile water, for final concentration of 10^4 cfu/g; attachment time of 1–1.5 h at 4*C was allowed. Treatment groups included: negative control, inoculated/untreated—no dip (T1); positive control, inoculated/treated with tap water (TW) (T2); SBS, 1%, 2%, and 3% in 300 mL TW (T3, 4, and 5, respectively); and SBS+200 ppm PAA, 1% SBS + PAA, 2% + PAA, and 3% + PAA in 300 mL TW (T6, 7, and 8, respectively). Inoculated WP for d 0, 1, and 3 were subjected to 15 s dip in respective solutions and were stored at 4*C until antimicrobial action was terminated by addition of neutralizing buffer.

After treatment, WP were allowed 2 min rest and rinsed in 150 mL neutralizing buffer for 1 min, after which WP was removed from each solution. Then 100 µL of rinsate solution was serially diluted for plating on XLT4 + NA agar for enumeration of NA resistant *S.* Typhimurium; plates were incubated aerobically for 24 h at 37*C. Log-transformed plate counts were analyzed using a randomized complete block design with a split plot over time. Using Fishers protected LSD, means were separated when $P \leq 0.05$. In this study, a day effect was observed ($P < 0.0015$). On d 3, there was a lower concentration of *S.* Typhimurium (3.44 Log cfu/g) compared with d 0 (3.91 Log cfu/g) and d 1 (3.90 Log cfu/g). No significant differences were found based on the mean log cfu/g of NA resistant *S.* Typhimurium per treatment ($P > 0.05$); however, the numerical concentration of *S.* Typhimurium by T5 (3% SBS) was less (3.54 Log cfu/g) than that of other treatments (3.64 Log cfu/g or greater). In conclusion, the overall level of *S.* Typhimurium was reduced over a 3-d refrigeration period; thus, potentially decreasing the risk of salmonellosis for consumers of processed poultry.

Key Words: *Salmonella* Typhimurium, sodium bisulfate salt, antimicrobial, processing, chicken wings

522P Genomic analyses of *Ornithobacterium rhinotracheale* clinical isolates and vaccine strains in US turkeys. E. Smith*, *University of Minnesota, St. Paul, MN.*

Ornithobacterium rhinotracheale (ORT) is a gram-negative bacterium causing primary or secondary respiratory disease in chickens and turkeys, as well as reduced weight gain, reduced egg production, and increased mortality. There are commercial vaccines available, as well as the option to use an autogenous vaccine produced from flock-specific bacterial cultures, but neither to date have demonstrated long-term effectiveness of preventing ORT infection in turkeys. There is a large knowledge gap regarding the molecular mechanisms of ORT, and a better understanding of the selective pressures on ORT pathogen populations in response to vaccination could highlight important genomic differences between clinical isolates and vaccine strains that could be limiting the efficacy of vaccination. The purpose of this study was to better understand ORT evolution in US turkeys using phylogenetic and gene content analyses of clinical isolates from commercial birds compared with vaccine strains used in their matched breeder flocks. Whole genome sequencing (WGS) was performed on ORT samples and vaccine strains collected from 3 major turkey companies (companies A, B, and C) from 2009 to 2018. WGS reads were mapped to reference genome ORT-UMN 88 to identify core SNPs across samples. A neighbor-joining phylogenetic tree was built in MEGA using a nucleotide substitution model and the Maximum Composite Liklihood method. R was used to generate a pairwise distance matrix and display hierarchical clustering among the ORT isolates and vaccine strains. Additionally, genomes were assembled for each isolate to identify gene content differences within and between phylogenetic clades using a pangenome approach involving Roary and Scoary. A total of 36,992 SNPs were identified from 80 ORT genomes. Phylogenetic analysis showed that isolates clustered by company and year. We identified some cases where clinical isolates from a company were different than the autogenous vaccine strain used at that time, suggesting that the vaccine strain is effective against certain clades but not cross-protective against diverse clades. In another case, a dominant monophyletic clade emerged where the company was vaccinating against this clade but was unsuccessful at eliminating clinical isolates from that clade. Gene content differences were identified from this clade that might help explain its enhanced virulence potential. The WGS approach enables high resolution tracking of clinical isolates related to vaccine use and may help to appropriately choose autogenous vaccine strains and determine times to switch vaccine strains. The genetic differences between the clusters also provide insight toward the evolution of highly virulent clades of ORT.

Key Words: *Ornithobacterium rhinotracheale*, turkey, genomics, vaccination, evolution

523P Development of in vitro models of competitive adhesion between probiotic *Lactobacillus* and human foodborne pathogens in poultry. M. Nash* and T. Duong, *Texas A&M University, College Station, TX.*

Lactobacillus species are widely used as probiotics because of their health promoting properties and are a potentially important alternative to the sub-therapeutic use of antibiotics in poultry production. Administration of probiotic *Lactobacillus* cultures to poultry has been demonstrated to improve pre-harvest microbial food safety by reducing gastrointestinal colonization of poultry by human foodborne pathogens. Although competition for adhesion sites on gastrointestinal tissues is thought to contribute to the competitive exclusion of pathogens by *Lactobacillus,* the mechanisms responsible for this functionality are not well understood. The goal of this study was to develop in vitro assays to investigate competition for adhesion to epithelial tissues between *Lactobacillus* species and human foodborne pathogens using the LMH chicken epithelial cell line. We have previously evaluated experimental factors including survival of bacteria in cell culture media, co-incubation times, and the number of post-incubation washes. The goal of this study was to evaluate the effects of the sequence of addition of bacteria and the relative ratios of *Lactobacillus* and pathogen added to chicken epithelial cells. Our results were used to develop a set of standardized experimental conditions to evaluate the ability of *Lactobacillus* cultures to inhibit binding of *Salmonella* from the chicken LMH cell line. By varying the sequence in which probiotic and pathogenic bacteria were added to the LMH cell line, we have developed assays to characterize the exclusion (*Lactobacillus* first), competition (simultaneous addition), and displacement (pathogen first) of pathogens from epithelial cells by *Lactobacillus* cultures. Exclusion of *Salmonella* from chicken epithelial cells by *Lactobacillus crispatus* ST1, *Lactobacillus crispatus* JCM 5810, *Lactobacillus gallinarum* JCM 8782, and *Lactobacillus gallinarum* ATCC 33199 was evaluated. *L. crispatus* JCM 5810 and *L. crispatus* ST1 significantly reduced adhesion of *Salmonella* by 80% and 50%, respectively ($P < 0.05$); while *L. gallinarum* ATCC 33199 and *L. gallinarum* JCM 8782 significantly reduced adhesion of *Salmonella* by 70% and 50%, respectively ($P < 0.05$). Future studies will extend our model to include other human foodborne (e.g., *Campylobacter*) and poultry pathogens (e.g., avian pathogenic *E.coli*, *Clostridium perfringens*), and candidate probiotic cultures from other genera (e.g., *Bacillus*, *Bifidobacterium*). Further development and validation of this model in live poultry and the use of targeted mutants of model *Lactobacillus* species including *L. crispatus* and *L. gallinarum* will contribute to a mechanistic understanding of probiotic functionality in poultry.

Key Words: *Lactobacillus*, probiotics, competitive exclusion, *Salmonella*

524P Effect of dietary raw potato starch levels on cecal microbiota in meat duck from 1 to 35d of age. S. Qin*, K. Zhang, X. Ding, J. Wang, S. Bai, and Q. Zeng, *Sichuan Agricultural University, Chengdu, Sichuan, China.*

The microbiota within hindgut plays an important role in protection from pathogens and maintain intestinal health. Currently, knowledge

about the impact of resistant starch diet on duck cecal microbiota is limited. In this study, Illumina Hiseq high-throughput sequencing and gas chromatographic techniques were used to investigate the effect of raw potato starch (RPS, RS2) levels on microbial composition and short chain fatty acids (SCFA) in the cecum of meat duck. A total of 360 1-d-old male ducklings were fed diets (8 pens of 15 ducklings, on each diet) containing different levels of RPS (0% (control), 12% and 24%) for 35 d. Subsequently, samples of cecal content (5 replicates were randomly selected from each group for microbial analysis) were prepared for examination. All data analyses were performed using R and Python. The model construction was performed by using R package Vegan. The results showed that 12% RPS group significantly elevated (1.7-fold) in Firmicutes compare with other 2 groups. At the genus levels, the relative abundances of *fecalibacterium*, *Ruminococcaceae UCG-014*, *Coprococcus*, *Subdoligranulum*, *Erysipelatoclostridium* and *Ruminococcus* increased significantly in 12%RPS-fed ducks ($P < 0.05$). Meanwhile, compare with 0% and 12% RPS groups, *Clostridium sensu stricto* and *Bifidobacterium* were higher in abundance, and *Oscillibacter* was reduced in the 24% RPS diet ($P < 0.05$). As for SCFA detection, acetate and propionate contents were greater when ducks fed the 12% RPS diet compared with control group ($P < 0.05$), and cecal butyrate was significantly higher with 12% and 24% RPS than that in ducks fed 0% RPS ($P < 0.001$). Accordingly, these results suggest that RPS not only alters the composition of the gut microbial composition but also modulates the metabolites-SCFA, which may further affect the hindgut health of the host.

Key Words: raw potato starch, caecal microbiota, short-chain fatty acids, meat duck

525P Correlating phenotype and genotype in turkey-derived *Lactobacillus johnsonii* to discover effective probiotic strains for use in commercial turkeys. A. Johnson*, J. V. Thomas, B. Weber, and T. Johnson, *University of Minnesota, Minneapolis, MN.*

Lactobacillus sp. have been widely used as probiotics in the poultry industry because they have been associated with gut health and increased growth performance. However, *Lactobacillus sp*. have been highly variable in their ability to modulate turkey gut health and growth performance. In this study we assess both phenotypic and corresponding genotypic properties of numerous strains of turkey gut derived *Lactobacillus johnsonii*, representing different phylogenetic backgrounds, with the goal of discovering effective probiotic strains for use in commercial turkeys. We used different in vitro assays to compare the probiotic potential (phenotype) of each strain of *L. johnsonii*. These assays include measuring each strain's acid tolerance, bile salt resistance, and ability to inhibit common poultry pathogens. We were able to determine that there was variability in assay performance between many of the strains ($P < 0.05$) with patterns observed related to *L. johnsonii* phylogenetic clade. To determine genotypic correlations with phenotypes observed in the different assays, we calculated the pan-genome for *L. johnsonii* using Roary on a large collection of isolates and then performed pan genome-wide association studies (pan-GWAS) using the software tool Scoary. Here we were able to find *L. johnsonii* genetic loci with high potential for being correlated with observed phenotypic probiotic assay performance. In conclusion, we were able to show that *L. johnsonii* strains vary phenotypically in probiotic performance assays, and these variations in phenotype correlated with genetic components using pan-genome analysis tools. In the future we hope to use these tools to quickly and easily screen potential probiotics which could then be used to enhance performance in commercial turkeys. Additionally we would like to develop a probiotic strain which contains multiple genes associated with increased probiotic performance.

Key Words: *Lactobacillus*, probiotic, bacteria, genome, turkey

526P Development of a high-throughput pathogen inhibition assay for screening potential probiotic *Lactobacillus* species. A. Wakil*, B. Weber, and T. Johnson, *University of Minnesota, St Paul, MN.*

The agar diffusion method is the current accepted pathogen inhibition assay, which is laborious and not high throughput. Our goal was to develop a high throughput pathogen inhibition assay. In initial development, we focused on comparing a disk diffusion assay and well-based methods using cell-free culture supernatants (CFCS) inoculated at different concentrations. CFCS for strains of differing inhibitory capability against avian *Escherichia coli* and *Salmonella* were generated by cultivating strains in MRS broth, followed by centrifugation to collect supernatant and subsequent filter sterilization. CFCS were then used directly or were pH-adjusted (pH 7). For disk diffusion, CFCS were added to 6 mm Whatman disks using several different techniques involving pipetting onto the disk or soaking the disk and adding the disk to Mueller-Hinton agar pre-streaked with the pathogen of interest. Plates were incubated overnight at 37°C and zones of inhibition were measured. The same CFCS were used for agar diffusion and well-based methods. In the well-based assays, supernatant was added to enteric culture and incubated at 37°C overnight. Using these approaches, we identified numerous lactobacilli strains that inhibited different pathogens. Many of these only inhibited pathogens when they were not pH-adjusted, indicating an inhibitory effect due to lactic acid production. However, our pathogens showed the capability to grow in acidic environments without the inhibitory supernatants present, indicating that the inhibition may be due to a pH dependent molecule. Both assays were effective at assessing pathogen inhibition. While the well-based method was used as a rapid screening technique during the initial experiments, it can be used as quantitative assessment of activity using a plate reader in future developments.

Key Words: *Lactobacillus*, pathogen inhibition, poultry, disk diffusion, supernatant

527P The effect of different bile salts on *Campylobacter jejuni* colonization in chickens. B. Alrubaye*, M. Abraha, M. Bansal, A. Almansour, H. Wang, B. Hargis, and X. Sun, *University of Arkansas, Fayetteville, AR.*

Campylobacter jejuni is a category B foodborne bacterial pathogen transmitted mainly from poultry. Currently few effective strategies are available to reduce *C. jejuni* poultry colonization. Bile acids were shown to reduce *C. jejuni*-induced intestinal inflammation. Here we hypothesize that microbial metabolite secondary bile acids reduce *C. jejuni* colonization in chickens. Broiler chickens were raised on floor pens and fed diets supplemented with 0 or 1.5 g/kg secondary bile acid deoxycholic acid (DCA), lithocholic acid (LCA), or urodeoxycholic acid (UDCA). Birds also orally gavaged with the primary bile acid cholic acid (CA) daily at 30 mg/kg body weight. The birds were orally infected with 10^9 cfu/bird *C. jejuni* UARK 101 at d10. The birds were sacrificed and cecal contents were collected at d2 and 18 post infection (PI) for enumerating *C. jejuni* colonization. Growth performance of body weight gain (BWG) and feed intake was measured at 14, 21, and 28 days of age. Interestingly, birds fed LCA showed significant reduction of BWG compared to infected control birds (0.83 vs. 1.47 kg/bird, $P = 3x10^{-7}$)

at day 28 of age, while primary bile acid CA and secondary bile acid UDCA and DCA did not significantly altered BWG. Importantly, at d18 PI only DCA diet reduced *C. jejuni* colonization compared to infected control birds ($2x10^6$ vs. $2.39x10^7$ cfu/g, $P = 0.03$), while primary bile acid CA and secondary bile acid LCA and UDCA did not significantly reduced *C. jejuni* colonization. In summary, DCA reduces *C. jejuni* chicken colonization compared to other primary and secondary bile acids and these findings may lead to antimicrobial free alternatives to control *C. jejuni* food contamination.

Key Words: *Campylobacter jejuni*, colonization, chicken, bile salts

528P Maturation of intestinal barrier function occurs around day 20 in developing chick embryo. T. Hamaoka*[1,2], J. Marshall[2], and A. Van Kessel[2], *[1]Calpis America Inc., Peachtree City, GA, [2]University of Saskatchewan, Saskatoon, SK, Canada.*

To determine if a nonvirulent *E.coli* strain would induce precocious development of the chicken digestive tract when administered into amniotic fluid in ovo, 3 trials were conducted. An *E.coli* strain (ECL01) was isolated from chicken intestinal tract and confirmed free of known *E.coli* virulence genes. Fertilized eggs (White Leghorn, University of Saskatchewan Poultry Centre, SK, Canada) were sanitized and incubated in HEPA-filtered incubators under standard conditions. In each trial, embryos (n=10) were assigned to experimental groups balanced by weight determined at E0. After treatment, all eggs were kept in HEPA filtered hatcher. To test dose response, sterile saline, 10^2,10^4,10^6 or 10^8 cfu/bird of ECL01 was inoculated (100 µL) in ovo on E17 to one of 5 treatment groups. To clarify effect of timing of inoculation, sterile saline or ECL01 (10^8 cfu/bird) was inoculated at E17, E18, E19 or E20. Hatchability and mortality were recorded at 23 days of incubation. In experiment 3, sterile saline or $1x10^2$ cfu/bird of ECL01 was injected in ovo in one of two treatment groups at E17. At pipping, the embryo was killed for measurement of body weight, organ size, ileal gene expression. All data were analyzed as a one-way ANOVA. Saline inoculation resulted in 100% hatch rate whereas embryos inoculated with all doses of ECL01 reached the pipping stage but failed to hatch. Only one embryo given 10^2 cfu hatched. This adverse effect of ECL01 was also observed when ECL01 was inoculated at E17, E18 and E19. However, when ECL01 was inoculated at E20, 100% of birds hatched and appeared normal and healthy. In experiment 3, embryos inoculated with ECL01 at E17 were studied in detail at the time of pipping. Inoculated embryos demonstrated decreased ($P < 0.0001$) yolk-sac-free body weight at pipping compared to the saline group associated with increased ($P < 0.001$) weight of the remnant yolk sac. Relative expression of interleukin-1, 6, 8 and toll-like receptor-2 were upregulated ($P < 0.05$) in ECL01 group. Tight junction protein Claudin (CDN) 1 was also upregulated ($P < 0.05$) whereas CDN4 tended to be decreased ($P < 0.10$) and CDN5 was decreased ($P < 0.05$). Non-pathogenic E. coli inoculated in amniotic fluid between E17 and E19 was lethal to chicks at piping whereas inoculation at E20 was not. Lethality was associated with reduced utilization of yolk sac nutrients, reduced yolk sac-free bodyweight, ileal expression of pro-inflammatory genes and altered expression of tight junction genes. Results indicate a maturation of intestinal barrier function normally occurs between E19 and E20.

Key Words: *E. coli*, in ovo, barrier function, chick quality

529P Effect of supplementation of an allochthonous probiotic bacterium, *Propionibacterium freudenreichii* ssp. *freudenreichii*, on the cecal microbiome of commercial turkeys challenged with multidrug resistant *Salmonella* Heidelberg. D. V. T. Nair*, T. Johnson, S. Noll, and A. K. Johny, *University of Minnesota, Saint Paul, MN.*

Multidrug resistant *Salmonella* Heidelberg (SH) has emerged as a successful colonizer in poultry and has been contributing significantly to foodborne outbreaks. We previously reported that *Propionibacterium freudenreichii* ssp. *freudenreichii* NRRL 3523 (PF), an allochthonous probiotic, was effective against SH colonization in the cecum of turkeys. In this follow up study, we investigated the effect of PF supplementation on the cecal microbiome of 7- and 12-week old commercial turkeys exposed to MDR SH challenge. Day-old turkey poults were allocated to 3 groups: negative control (NC; -PF, -SH), SH control (SC; -PF, +SH) and the test group (PFS; +PF, +SH). Turkeys (7-week study – 8 turkeys/group, 2 experiments; 12-week study – 10 turkeys/group, 2 experiments) were supplemented with PF in water (10^{10} cfu/ml) until 7- or 12-weeks of age. At 6th or 11th week of age, turkeys were challenged with SH at 10^6 or 10^8 cfu/bird, respectively. After 2- and 7 d post-challenge, cecal samples were collected and 16S rRNA gene profiling was performed using Illumina MiSeq. The α diversity and the effect of treatments on the bacterial community composition were analyzed using one-way ANOVA and PERMANOVA, respectively. The species richness and evenness (Shannon diversity index) was similar among the groups. Using β diversity metrics, samples were clustered by groups, and they were significantly different from one another ($P < 0.05$). Firmicutes were the predominant phylum in the turkey cecum evenly distributed among the groups except on wk 12 where the relative abundance was significantly higher in PFS ($P = 0.02$). The SH challenge resulted in the higher abundance of *Streptococcus*, *Weissella,* and *Turicibacter* in the SC group ($P < 0.05$), microflora that may be associated with inflammation, indicating alterations in the cecal microbial populations due to SH challenge. Supplementation of PF increased the relative abundance of carbohydrate fermenting and short-chain fatty acid (SCFA) producing genera such as *Romboutsia*, *Ruminococcaceae*_NK4A214_group, *Ruminococcaceae*_UCG.010, *Lactobacillus*, *Leuconostoc, Lactococcus,* and *Butyricicoccus* in the PFS groups compared with SC and NC ($P < 0.05$), indicating promotion of potentially beneficial bacteria. In addition, PF supplementation resulted in significantly reduced abundance of *Streptococcus*, *Weissella,* and *Turicibacter*, compared with SC, indicating potential restoration of cecal microbiota by PF. In conclusion, supplementation with PF shifted the microbiome to more beneficial bacteria during SH challenge and decreased bacteria associated with inflammation (MIN-16–102).

Key Words: *Propionibacterium freudenreichii*, allochthonous probiotic, *Salmonella* Heidelberg, cecal microbiome

530P Evaluation of different poultry serovars and strains of *Salmonella enterica* for cellulose-based biofilm formation. Z. Shi*, K. Feye, and S. Ricke, *University of Arkansas, Fayetteville, AR.*

Salmonella is a leading cause of foodborne illnesses worldwide and exhibits numerous virulence mechanisms contributing to its pathogenesis, with poultry being a major reservoir. This includes the ability to form pellicle (air-liquid) and bottom (liquid-solid) biofilms, which contributes to the pathogen's antimicrobial resistance and its ability to persist in poultry processing environments. The 2 structures responsible for biofilm formation are curli and cellulose production. The response of the master regulators of curli (*csgD*) and cellulose (*bcsA*) actively guide biofilm formation. It is unknown if this regulation is the same across all 2,500 serovars of *Salmonella*. The purpose of this study was to determine if pellicle formation has serovar and strain specific transcription regulator gene expression signatures. Poultry isolates *S.* Kentucky (ARS44,ARS45), Heidelberg (ARI14,ARI15,ARI16) and Enteritidis

(ARS46) were evaluated for the fold-change difference in *csgD*/*bcsA* gene expression. Briefly, 50mL of salt-free Luria-Bertani media were inoculated with overnight cultures of their respective *Salmonella* serovars and strains, grown at room temperature (4 d). Samples were taken below the meniscus at time points D0, D1, D2, and D3, and below the pellicle at D4. The RNA was extracted using the Qiagen RNeasy kit. Reverse Transcription Quantitative PCR (RT-qPCR) was performed on the extracted RNA using the Verso 1-Step RT-qPCR kit with primers targeting *bcsA* and *csgD* expression with *rsmC* acting as an internal control. Normalized CT values were imported into SAS and analyzed using proc genmod and adjusted using Tukey's adjustment for multiple comparisons. There was a significant effect of day and gene expression across all serovars and strains ($P < 0.001$). Temporal differences emerged among all strains, although the pattern of variation showed gene, strain and serovar dynamics. For example, *S*. Enteritidis, ARS46, showed significant increases in *csgD* gene expression by a 1.5-fold decrease in expression from D0 and D1 ($P < 0.05$); however, there was no difference in gene expression between D1-D2, D2-D3, and D3-D4. There are significant differences in *csgD* expression compared with *bcsA* with an average difference in gene expression of approximately 1.5-fold on D2, D3, and D4 ($P < 0.05$). There was no difference between the 2 genes on D0 and D1. These dynamics were not conserved across strains nor serovars. It appears that the regulation of pellicle formation is serovar and strain specific, exhibiting significant transcription factor dynamics across time. This knowledge may be helpful in understanding serovar prevalence and for the development of more targeted treatments of biofilms on poultry processing surfaces.

Key Words: *Salmonella*, biofilm, pellicle, cellulose, RT-qPCR

531P Comparative genome analyses of avian pathogenic *Escherichia coli* (APEC) from commercial turkey production in the United States. N. Jahan*[1] and T. Johnson[2], [1]*University of Minnesota, St-Paul, MN.*

Avian pathogenic *E. coli* (APEC) cause colibacillosis, a disease of severe economic significance to all poultry producers worldwide. This disease is difficult to control in part because of the diversity of APEC populations in the field. In numerous previous studies, the most common APEC serogroups and virulence factors have been thoroughly defined. However, study of APEC in commercial turkey production are lacking compared with broiler production. Additionally, few studies have examined the microevolution of APEC within vertically integrated systems using high resolution genomic approaches. The purpose of this study was to investigate the phylogenetic relatedness of APEC within and between 3 major commercial turkey production companies in the US, and to better describe the landscape of APEC in commercial turkey production as compared with broiler production. We obtained 300 clinical *E. coli* isolates from 3 major commercial turkey companies from 2016 to 2018, representing hundreds of farms across the United States. Whole genome sequencing was performed to assess virulence factors, antibiotic resistance genes, plasmid types, serotypes, and sequence types. Phylogenetic relatedness of APEC was examined using whole genome single nucleotide polymorphisms, comparing evolution of APEC across and within companies. Pangenome analyses were conducted to support this analysis. Results indicate that while a diverse collection of clones were identified, several dominant ST types and serogroups represent the vast majority of isolates analyzed. These dominant clones included ST131, ST69, and ST117. As previously determined in broilers, possession of ColV plasmid and associated genes were a defining trait of turkey clinical isolates, but their prevalence among contemporary turkey isolates was much higher than previously reported in broilers. Multidrug resistance was common in the isolates, and conferred by transmissible plasmids such as Inc.A/C and Inc.I1. Collectively, such information provides the basis for the development and enhancement of strategies to control APEC infections in commercial turkey production.

Key Words: turkey, APEC, colibacillosis, virulence, phylogenetic

532P Modeling and kinetic characterization of the cross-contamination of *Salmonella* in poultry chilling process. W. Wang*[1], X. Xiao[2], R. Wang[1], L. Cooney-Kelso[1], W. Wang[3], M. Kidd[1], and Y. Li[1], [1]*Univerisity of Arkansas, Fayetteville, AR,* [2]*Zhejiang University, Hangzhou, Zhejiang, China,* [3]*Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang, China.*

Salmonella cross-contamination in the chilling process of poultry carcasses is a food safety issue. The objective of this study was to characterize the survival and inactivation of *Salmonella* in poultry chilling process and develop a predictive model for cross-contamination of *Salmonella* in chiller water and poultry carcasses. To study the inactivation of *Salmonella*, 20 chicken breast meat samples were prepared in each of the triplicate trials, and 10 of them were inoculated with *Salmonella* Typhimurium to achieve an initial population of 10^5 cfu/g. Five inoculated samples and 5 uninoculated samples were put into different sides of a filtered stomacher bag filled with chlorinated water, and incubated in a chiller water bath. Treated chicken breast meat samples and 5 water samples were plated on trypticase soy agar and xylose lysine tergitol-4 agar to determine the number of *Salmonella* cells, while the rest of inoculated and uninoculated samples were carried out as positive and negative controls, respectively. The difference of *Salmonella* concentration on chicken breast meat samples before and after the treatment were determined using *t*-test. With total chlorine concentration of 50 ppm (free chlorine concentration > 10 ppm), chiller water temperature of 4°C, and exposure time of 50 min, a *Salmonella* reduction of 0.98-log cfu/g ($P < 0.05$) was observed on inoculated chicken breast meat samples, while 2.64-log cfu/g *Salmonella* cells ($P < 0.05$) were transferred onto uninoculated samples through chiller water. Post-treatment chlorinated water was proven to be pathogen-negative, however, dynamic total chlorine concentration dropped to ~10 ppm, with free chlorine concentration < 1 ppm. The preliminary result indicated limited efficacy of sodium hypochlorite against cross contamination of *Salmonella*, due to the protective effect of chicken tissue, and dynamic reduction of free chlorine. A predictive model is being developed to describe the inactivation kinetics and major parameters (including total/free chlorine concentration, exposure time, temperature and pH), and validated with the tests on chicken carcasses.

Key Words: *Salmonella*, cross-contamination, inactivation, chilling process, modelling

533P A microbial survey of turkeys from hen to hatch. S. Anderson*, E. Hutchison, E. Vang, R. Wujek, and T. Rehberger, *Arm and Hammer, Waukesha, WI.*

A concern among the turkey industry is the pathogen challenge facing day-old poults. Previous research has shown that there is limited vertical transmission of beneficial bacteria like lactic acid bacteria (LAB) and yet, pathogens like avian pathogenic *E. coli* (APEC) appear to be vertically transmitted from the breeder hens. The purpose of this study was to determine the source of bacteria in the gastrointestinal tract (GIT) of day-old poults. The microbiota of 2 turkey egg clutches were studied at multiple points in the post fertilization process; pre-incubation eggs before and after H_2O_2 rinse, embryo GITs, embryo yolks and a shell

swab and day-old poult GITs and yolks along with the breeder hen GITs and oviducts from the same flocks. Enumeration of LAB, APEC and *Clostridium perfringens* were done on all the samples; LAB isolates were identified using 16S rRNA sequencing and *C. perfringens* were typed by random amplification of polymorphic DNA. Enumeration data showed that APEC levels in day old poults were below detectable levels which is contrary to previous observations within this same company, but is consistent with the flock to flock variation commonly observed. Although 2 C. perfringens isolates from embryo GITs were similar to populations found in the hens, the majority of the *Clostridium* populations found in day-old poults were not similar to the predominant isolates from the breeder hens. There was a numerical decrease in LAB levels between the pre and post H_2O_2 rinsed eggs, however this decrease was not statistically significant ($P = 0.2145$). The predominant bacteria isolated from lactobacilli agar plates in the GIT of day-old poults were *Enterococcus faecalis* (70%) and *Staphylococcus aureus* (25%). *S. aureus* isolates were not detected in the breeder GITs or oviducts, but were present on the shell both at pre-rinse (6%) and after incubation (38%). *E. faecalis* were detected in the breeder GITs (7%) and oviducts (7%) and were predominant on the shell pre-rinse (26%) and detected at a lower level after incubation (15%). No *E. faecalis* or *S. aureus* were detected in the GITs or yolk sac of the embryos. *Bacillus licheniformis* was predominant in the GIT (62%) and yolk sac (42%) of the embryos but comprised less than 5% in the day-old poult. According to this study, the predominant bacteria detected in day-old poults is environmentally transmitted via the outer egg shell.

Key Words: turkey, microbiota, embryo, APEC, *Clostridium*

534P Effect of lemongrass essential oil on *Salmonella* Heidelberg colonization in commercial broiler chickens. G. Dewi*, D. V. T. Nair, S. Manjankattil, C. Peichel, J. Langlie, and A. K. Johny, *University of Minnesota, Saint Paul, MN.*

Salmonella Heidelberg (SH) is a highly invasive human pathogen for which broilers serve as reservoir hosts. Recent reports indicate that SH has been frequently isolated from poultry and their products. Therefore, control of SH populations in broilers is necessary as SH colonization increases the risk of carcass contamination during processing. Among many alternatives investigated these days, essential oils are emerging as potential alternatives to antibiotics, contributing to improving the preharvest safety of broilers. We previously found that lemongrass essential oil (LGEO), a GRAS oil extracted from *Cymbopogon spp.*, was effective against SH in chicken drinking water in vitro. In this study, we investigated the efficacy of LGEO as an antimicrobial supplement either through feed or drinking water to reduce SH colonization in broilers. A total of 4 challenge studies, 2 each for feed- (10 birds/group; 30 birds/study; 2 studies) and water- supplementation (3 birds/group; 9 birds/study; 2 studies), were conducted. The treatments included a negative control (NC), *Salmonella* control (PC), and LGEO supplemented group (EO). All birds, except those in NC groups, in the feed and water supplementation studies were challenged with 5.2- and 3.8 $\log_{10}$ cfu SH (2014 Tennessee correctional facility outbreak strain), at wk 2 and wk 4, respectively, by crop gavage. In the feed study, LGEO was added to the feed at 1% from d 1 through wk 3, whereas in water supplementation study the birds received LGEO at 0.5% through water only after the SH challenge. All birds were euthanized after 7 d post-inoculation (3rd and 5th week for feed and water studies, respectively), and the surviving SH populations in the cecum were enumerated. Number of birds in the challenge models was determined using power analysis and SH colony counts were logarithmically transformed before statistical analysis. Mixed effects model was performed using lmerTest package of R and significance set at $P < 0.05$. For both feed studies where 1% LGEO was supplemented through feed, no significant reduction in SH populations was observed between the PC and EO groups ($P > 0.05$). Likewise, when supplemented through drinking water, 0.5% LGEO resulted in no significant reduction in the first study but a 2.4 $\log_{10}$ cfu/g reduction in SH populations was observed in the second study ($P < 0.05$). The results indicated that LGEO supplementation through feed may not be effective against SH colonization potentially due to its interaction with feed ingredients, but could be more potent when supplemented through drinking water. Final body weight gain was similar across treatments. Additional studies examining LGEO supplementation through water in broilers are underway.

Key Words: *Salmonella* Heidelberg, lemongrass essential oil, cecal colonization, preharvest safety, broiler chicken

535P The effect of a *Bacillus* DFM program on avian pathogenic *E.coli* populations within the US turkey industry. R. Wujek*, E. Hutchison, A. Smith, S. Anderson, and E. Vang, *Arm and Hammer Animal Nutrition, Waukesha, WI.*

Systemic infection by avian pathogenic *E. coli* (APEC) can cause colibacillosis in turkeys, which is often fatal and imparts a significant economic burden on the turkey industry. Increased regulatory and consumer pressure for the reduction of antibiotic use has been the driving force for finding alternative forms of enteric pathogen control. Direct-fed microbials (DFMs) have been shown to be an effective alternative to antibiotics for controlling APEC. While some DFMs vary in effectiveness from company to company, Arm and Hammer Animal Nutrition (AHAN) uses a customizable program to tailor *Bacillus* strain combinations to meet the specific challenges of each company. Once a company is on product, follow up surveys are conducted to monitor APEC population levels and changes in diversity. Gastrointestinal tracts (GITs) from farms were processed and serial dilutions were plated to differntial media to enumerate *E. coli*. Representative isolated colonies were subject to a multiplex PCR. *E. coli* was considered to be APEC if it possessed 2 or more of the following virulence genes, *iroN, ompT, hlyF, iss*, and *iutA*, detected by multiplex PCR. This study analyzed the APEC levels of 1,740 non-diseased turkey GITs to determine the effect of the AHAN DFM program. Of the 1,740 turkeys sampled from 18 companies, 1,111 were on product and 629 were not on product. The average level of APEC for turkeys from all birds fed AHAN *Bacillus* product was 1.08E+05 cfu/g whereas the average level of APEC in the intestinal tract of non-treated turkeys was 1.50E+06 cfu/g; these differences were significant (*t*-test of log-transformed data, $P < 0.05$). Birds were also compared by 2-week time points (0–2, 3–4, 5–6, 7–8, 9–10, 11–12, and > 12 wks). APEC levels were reduced in AHAN *Bacillus*-fed birds at all ages except at 5–6 wks, but this reduction was only significant at 0–2 wks of age. This large difference in young birds may be explained by the beneficial impact of the *Bacillus* DFM in the largely unestablished microbiota of poults. In conclusion, the AHAN DFM program resulted in a log and a half reduction in intestinal APEC in turkeys across 18 companies.

Key Words: *E. coli*, *Bacillus*, DFM, APEC, colibacillosis

536P Proteomic analysis of viable but nonculturable (VBNC) state of *Campylobacter jejuni*. D. Samarth*, R. Liyanage, J. Lay Jr., and Y. M. Kwon, *University of Arkansas, Fayetteville, AR.*

Campylobacter jejuni is a zoonotic pathogen and also the major cause of foodborne diarrheal illness in humans. Under stress conditions, *C.*

jejuni is reported to have a change in morphology (Spiral to coccoid) and metabolic status. This condition, termed as Viable but non culturable forms (VBNC), still remains a subject of much debate. It is also unclear whether *C. jejuni* cells under this condition retains virulence, and remains transmissible to humans. To gain more insight into this state, we performed proteomic studies by analyzing *C. jejuni* cells re-suspended in freshwater microcosm water and recovered at different time intervals. Overnight culture of *C. jejuni* on Mueller Hinton (MH) agar was re-suspended in microcosm water and incubated at 25°C for 30 d without maintaining microaerophilic conditions, and the samples were collected at 1 d, 3 d, 7 d, 15 d, and 30 d. The *C. jejuni* cells became unculturable after 3 d on MH plate under the condition. Samples were used for SDS-PAGE-based protein extraction. The extracted proteins were subjected for proteomic analysis using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Total number of *C. jejuni* proteins identified were 459. The number of identified proteins declined over the time period. Clustering analysis according to the patterns of dynamic changes over time grouped all proteins into 6 different clusters. The proteins in Cluster 4 (39 proteins) and Cluster 5 (44 proteins) showed consistently increasing trends of protein abundance over the time. After functional enrichment analysis of these proteins we found that Cluster 4 and 5 contain proteins, which play role different in biological and cellular pathways including translation, nucleic acid and RNA binding. Cluster 5 comprise the proteins such as VirB4 and ComEA which play role in virulence whereas cluster 4 contain CheW which play role in chemotaxis. By the means of this study we attempt to speculate the changes in protein levels in *C. jejuni* cells over the experimental time period. Proteomic analysis of VBNC will provide new insights into about *C.jejuni* physiology.

Key Words: *Campylobacter*, VBNC, viable but nonculturable, proteomics, survival

537P The feasibility of using multimode hyperspectral imaging technology to detect *Salmonella enterica* in pet food. E. Monu*[1], D. Rosen[2], R. Hellberg[2], F. Vasefi[3], D. Farkas[3], and R. Norton[1], [1]*Auburn University, Auburn, AL,* [2]*Chapman University, Orange, CA* [3]*SafetySpect, Sherman Oaks, CA.*

Salmonella has caused several outbreaks in human food, pet food and animal feed. There are well established traditional culture based methods for the enumeration of bacterial pathogens from food and animal feed. But these methods are laborious and time intensive, leading researchers to continue to develop new and innovative rapid detection techniques. Multimode hyperspectral imaging (MHSI) is such a method. Food is multicomponent in nature, which means its reflectance/fluorescence spectra are complex and chemometric methods using multivariate analysis can be used to extract contaminant specific information in samples. MHSI overcomes the limitations of single spectral methods, combining reflection and fluorescence with UV Raman spectroscopy. By varying the applied excitation and detection wavelengths and measuring the reflectance and fluorescence emission properties of a food sample, it is possible to fine-tune algorithms for specific foods and contaminants. Objective: The objective of this study was to evaluate the potential of the SafetySpect MHSI equipment to detect *Salmonella enterica* in inoculated pet food. Dog food was inoculated with *Salmonella* Infantis ATCC 51741 that had been grown in Tryptic Soy Broth (TSB) at 37°C to a population of 10^6 cfu/mL. Seven 25 g samples of dog food were weighed out, spot inoculated with *S*. Infantis for a final inoculum of 10^1 – 10^6 cfu/g and allowed to dry in a biosafety cabinet for 2 h. Imaging was conducted in sterile glass petri dishes in a tabletop system with UVA (371 nm, FWHM 16nm, power =91.7mW/cm^2) and blue/violet (418 nm, FWHM = 21nm, power = 38.9mW/cm^2) LEDs for fluorescence excitation and white LEDs (power = 35.8mW/cm^2) for reflectance. The system analyzed samples at a bandwidth of 450 – 800 nm within microseconds and achieved ca. 90% illumination uniformity, 89 µm spatial resolution and 8 nm spectral resolution. As this was a pilot study to determine regions of interest (ROIs) in the output, no statistical analysis was conducted. Future trials will evaluate several strains of *Salmonella* for comparison. Five ROIs were determined and the final reflectance measurements for each pet food sample were established using the median measurements from these 5 ROIs. Reflectance and fluorescence emissions correlated with increased *S*. Infantis contamination. The evaluated multimode hyperspectral imaging technology shows potential as a novel rapid detection technique for *Salmonella* in low water activity pet food.

Key Words: rapid detection, *Salmonella*, hyperspectral imaging, food safety, pet food

538P A broad view of how APEC from yolks and gastrointestintal tracts of day-old chicks compare across different complexes within a company. E. Vang*[1], E. Hutchison[1], S. Anderson[1], R. Wujek[2], T. Rehberger[1], and A. Smith[1], [1]*Arm & Hammer Animal Nutrition, Milwaukee, WI,* [2]*Arm & Hammer Animal Nutrition, Waukesha, WI.*

Avian pathogenic *Escherichia coli* (APEC) is a highly ubiquitous pathogen present throughout the life of commercially raised chickens. It has been associated with high mortality of chicks in the first weeks of life, which places a significant economic burden on the poultry industry. This study was carried out to determine the variation of APEC populations of day-old-chicks (DOC) between complexes within a single poultry company, and to examine the relationship of APEC populations between yolks and gastrointestinal tracts (GIT). A total of 179 broiler DOC GITs and their corresponding egg yolks were sampled from 3 different complexes (A, B, and C) within a single US broiler company. GITs and yolks were enumerated for *E. coli*, and up to 5 colonies from each sample were isolated and cultured for down-stream characterization. Five APEC virulence genes (*hlyF*, *iroN*, *iss*, *iutA*, and *ompT*) were used to distinguish APEC from non-APEC isolates and random amplified polymorphic DNA (RAPD) typing was used to further characterize the genotype of confirmed APEC strains. In total, 630 *E. coli* isolates were isolated from all the GITs and yolk samples of which 567 (90%) were found to be APEC. The GITs yielded 321 APEC (57% of the total APEC) while yolks yielded 246 APEC (43% of the total APEC). There was bird to bird variation throughout each complex with APEC levels ranging from below detectable limits to greater than 2.0×10^8 cfu/g of tissue. Overall, APEC levels of GITs were significantly higher compared with yolks across all complexes (*t*-test of $\log_{10}$ transformed data, $P < 0.05$). Genotyping showed that 88% of all the APEC isolates were common to all complexes however, complex B possessed APEC populations that were not associated with any other complex. In conclusion, these findings suggest that APEC populations are present across poultry complexes, although there are also complex-specific populations. In addition, while similar populations of APEC were detected between yolk and GIT, yolks had consistently lower detectable levels of APEC, indicating that GITs may be a better tissue source for characterizing APEC populations.

Key Words: APEC, RAPD, virulence, genotype, variation

539P Serotype, antimicrobial susceptibility and genotype profiles of *Salmonella* isolated from duck farms and slaughterhouse in Shandong province, China. S. Sun, J. Yang, and Z. Ju*, *Shandong Agriculture University, Taian, Shandong, China.*

This study investigated the serological prevalence, antimicrobial resistance, characterization of class 1 integrons and genetic diversity of *Salmonella* isolated from duck farms and slaughterhouse in Shandong province, China. A total of 49 *Salmonella* strains were collected out of 2340 samples (including drinking water and fodder samples, water samples from duck-washing pools, cotton swabs from table surface in slaughterhouses and embryo, leg meat, feces, liver, intestine and spleen samples of ducks) from 4 duck farms and one duck slaughterhouse in Tai'an and Jinan, 2 important cities of Shandong province. Among the isolates, *S*. Enteritidis (23/49, 46.94%) and *S*. Typhimurium (6/49, 12.24%) were the most prevalent, and high resistance rates were detected for erythromycin (48/49, 97.96%) and nalidixic acid (46/49, 93.88%). A total of 34.69% (17/49) strains were found carrying class 1 integrons, containing gene cassettes *aadA7+aac3-ld* (15/17) and *aadA5+dfrA17* (2/17). Eleven different kinds of resistance genes were detected while bla_{TEM} (36/49, 73.47%) was the most prevalent one and followed by *sul2* (14/49, 28.57%). Thirteen virulence genes were tested, all the strains (49/49, 100%) carried *invA, hilA* and *sipA* and most strains carried *stnP1* (45/49, 91.84%) and *ssrA* (45/49, 91.84%). MLST result showed that STs were related to serovars, while ST11 was the most prevalent ST (20/49, 40.82%), followed by ST2441 (10/49, 20.41%). The PFGE patterns were diverse and revealed the phenotypical and genotypical variety in the strains collected from different sampling sites, while some strains may derived from same origins. The presence of *Salmonella* infections among duck farms revealed that ducks could also be potential reservoirs for *Salmonella*. The high resistance rates against commonly used antimicrobials indicated the need for more reasonable use of antimicrobials.

Key Words: *Salmonella*, antimicrobial resistance, duck, MLST, PFGE

617P Recovery of *E. coli* from the yolk sacs of chickens incubated with light in combination with sanitation with lysozyme. Nilakshi Abeysinghe*, Eilish Conners, Xujie Li, Janice MacIsaac, Bruce Rathgeber, *Dalhousie University*.

Hatcheries are eager to find alternatives to antibiotics to control bacteria during incubation. The aim of this study was to evaluate an alternative sanitizer in combination with light during incubation. Before incubation 144 broiler and 160 layer hatching eggs were exposed to an overnight culture of naladixic acid resistant *E.coli*. The eggs were fumigated with either 3% lysozyme for 10 min or water for the same time. The eggs were randomly assigned to 8 incubators in equal numbers (20 layer eggs and 18 broiler eggs). Four incubators were illuminated with dim to red LED lights for 12 h each day and the others remained in the dark. On day 18 of incubation, eggs were transferred to hatching trays with half of each group transferred to an incubator with the same lighting treatment. Two incubators from each lighting treatment were fumigated with either water or 3% lysozyme. At 512 h of incubation the chicks were euthanized and yolk sacs were harvested. The yolk samples were diluted and plated on EMB agar in duplicate to detect the presence of naladixic acid resistant and non resistant *E.coli*. The average incidence of samples from each incubator was determined and Proc Mixed was used to determine the impact of treatment combinations on the presence of the bacteria in the yolk sac. Hatchability was not influenced lights or sanitation however broiler hatchability was greater than layer eggs ($P < 0.05$). There was no significant difference in the incidence of *E.coli* in eggs fumigated with 3% lysozyme or water. The layer chicks exposed to red light possessed a much higher incidence of *E.coli* (78%) than the layers incubated in the dark (17%). The incidence of resistant *E. coli* was also higher for layers from light (51%) compared to the dark (17%). The incidence of non resistant *E. coli* was not different for broilers subject to different incubation conditions but the resistant *E. coli* were not found from the yolk sacs of broilers incubated in the light compare to the dark (21%). The results indicate that prevention of bacterial contamination is possible by controlling environmental factors such as light exposure.

Key Words: incubation, photoperiod, *E. coli*, lysozyme, naladixic acid

540P Distribution of cells expressing SGLT1 mRNA in the chicken yolk sac and small intestine at different developmental ages. H. Zhang* and E. Wong, *Virginia Tech, Blacksburg, VA.*

SGLT1 is one of the most important transporters for mediating the uptake of glucose. In mammals, SGLT1 mRNA is expressed in the intestinal enterocytes and SGLT1 protein is localized at the brush border membrane. In chickens, SGLT1 mRNA was shown to be expressed in both the yolk sac and small intestine. The objective of this study was to identify cells in the yolk sac and small intestine that expressed SGLT1 mRNA during the transition from late embryogenesis to early post-hatch. SGLT1 mRNA was detected by chromogenic in situ hybridization. The stained images showed that expression of SGLT1 mRNA in yolk sac epithelial cells was low from embryonic d 11 to 17, peaked at embryonic d 19 and declined at day of hatch. In the small intestine, cells expressing SGLT1 mRNA were present not only along the intestinal villi but also in the crypts, with greater expression of SGLT1 mRNA in the epithelial cells that line the villus than in the stem cells located in the crypts. The latter result suggests that intestinal stem cells utilize glucose. Expression of SGLT1 mRNA in the intestine increased from embryonic d 19 to day of hatch and then maintained a high level of expression from d 1 to d 7 post-hatch. For both the yolk sac and small intestine, the temporal pattern of SGLT1 mRNA expression detected by in situ hybridization was consistent with the pattern previously reported by quantitative PCR.

Key Words: SGLT1, in situ hybridization, yolk sac, small intestine

541P Neuropeptide Y is expressed in chicken liver and regulates hepatic lipogenesis. C. Shawhan*, E. Greene, and S. Dridi, *University of Arkansas, Fayetteville, AR.*

Although poultry genetic selection has allowed spectacular progress in feed efficiency and muscle development, it was accompanied with undesirable changes such as muscle myopathy and fat accumulation. Neuropeptide Y (NPY), mainly expressed in the hypothalamus, has been linked to increased food intake and lipid metabolism. More recently, NPY has been found in peripheral tissues, but its role is still unclear. This study aimed, therefore, to characterize the hepatic NPY system and to determine its effect on hepatic de novo fatty acid synthesis (lipogenesis). For characterization, total RNA was extracted from liver tissues taken from 4-week old broilers raised under standard conditions, spontaneously immortalized chicken embryonic liver (sim-CEL) cells, and Leghorn male hepatoma (LMH) cells. Hypothalamic tissues were used as a positive control. The expression of NPY and its related receptors was determined by PCR. To determine the role of NPY in the liver, sim-CEL cells were treated with 0 or 100 nM of recombinant NPY for 24 h. Expression of lipogenesis-target genes and proteins were determined by RT-qPCR using $2^{-\Delta\Delta CT}$ method and Western blot, respectively. Data were analyzed using a Student's *t*-test and results were considered statistically significant when $P < 0.05$. Conventional PCR revealed that NPY and its related receptors 2, 5, and 7 are expressed in chicken liver tissue, sim-CEL cells and LMH cells. NPY receptors 4 and 6 are expressed in sim-CEL cells and LMH cells. NPY-R1 is expressed in LMH cells. NPY treatment significantly upregulates its own system's expression as well as the expression of fatty acid synthase (FASN) and ATP citrate lyase (ACLY), which are rate limiting in fatty acid synthesis. Together, these results indicate that NPY is expressed in the avian liver, and it may play a role in regulating lipogenesis. Since the liver is the primary site for de novo fatty acid synthesis in avian species, further investigation of NPY expression in the liver could lead to a better insight of how fat metabolism is regulated.

Key Words: neuropeptide Y, liver, lipogenesis, sim-CEL, chicken

542P Dynamin plays a crucial role in lipids absorption in endodermal epithelial cells of avian yolk sac membrane. C. T. Tung*, H. J. Lin, Y. J. Chen, C. C. Huang, and S. T. Ding, *National Taiwan University, Taipei, Taiwan.*

Endodermal epithelial cells (EECs) within yolk sac membrane (YSM) are liable for the absorption of yolk lipids which packaged mainly in very low-density lipoprotein (VLDL) during avian embryonic development. Dynamins (DNMs) serve crucial roles in Clathrin-mediated endocytosis, including uptake of VLDL via several VLDL-receptors. However, during embryonic development, the role of DNMs-related endocytosis for uptaking VLDL in EECs is still unclear. In this study, we utilized Japanese quails as an animal model and isolated EECs from YSM to evaluate DNM-related VLDL uptake. The mRNA expression patterns of DNMs in YSM during development were examined. Expression of DNM1 reached a peak at d 10 of incubation, and was significantly different from d 2 of incubation ($P < 0.05$, by one-way ANOVA). To evaluate DNM-related VLDL uptake, the isolated EECs of incubation d 5 were treated with DNMs inhibitor Dynasore (80 μM) for 10, 20, and 30 min and then supplemented with 800, 1050, and 1300 μg/mL of fluorescence-labeled yolk VLDL (Dil-yVLDL) for 2 h. Treatment of Dynasore for 30 min dramatically blocked Dil-yVLDL absorption in EECs. Furthermore, EECs were treated with DNMs activator S-Nitroso-L-glutathione (GSNO, 100 μM) for 30 min and then supplemented with 1000 μg/mL of Dil-yVLDL for 5, 10, 15, and 20 min. Results showed that treatments of GSNO for 15 and 20 min significantly increased Dil-yVLDL uptake in EECs, respectively, compared with the control group. To conclude, these results showed elevated DNM expression in the mid- to late- term of incubation, and DNMs are indispensable in assisting VLDL uptake of EECs during quail embryonic development.

Key Words: avian embryonic development, dynamin, endodermal epithelial cells, very low density lipoprotein, yolk sac membrane

543P Differential response of the cecal tonsil to aflatoxin B1 in domesticated and wild turkeys. K. Reed*[1], K. Mendoza[1], and R. Coulombe[2], [1]*University of Minnesota, St. Paul, MN,* [2]*Utah State University, Logan, UT.*

Domesticated turkeys (*Meleagris gallopavo*) are especially sensitive to the hepatotoxic effects of the food-borne mycotoxin aflatoxin B1 (AFB_1). Studies have shown that domesticated turkeys lack functional hepatic GST-mediated detoxification. Wild turkeys have GST activity and are therefore more resistant to the hepatotoxic effects of AFB_1. Whole genome RNA expression (RNA-seq) studies have found gene dis-regulation in response to AFB_1 insult with significant association of phase I (modification) and phase II (conjugation) metabolism genes and genes involved in cellular regulation, modulation of apoptosis, and inflammatory responses. Given the negative hepatic effects of AFB_1, this study was designed to examine the effects of AFB_1 on the turkey gut, specifically focusing on the cecal tonsil, representing both the digestive system and gut-associated lymphoid tissue. Two week old poults (domesticated and eastern wild subspecies, *M. g. silvestris*) were fed either a diet containing 320 ppb AFB_1 or control diet for 14 d and

cecal tissue harvested for RNA-seq. Indexed libraries (n = 16) were constructed from isolated RNA, multiplexed, pooled and sequenced (101-bp paired-end reads) to produce 9.8M to 14.2M reads per library (average 12.7M). Sequence reads were mapped to the turkey genome and empirical analysis of differential gene expression was performed on normalized mapped read counts. In the domesticated turkeys, significant differential gene expression was observed for 11,237 genes (FDR p value <0.05) in birds exposed to AFB_1 versus control-fed birds. The number of significant genes (703) was considerably fewer for the AFB_1-treated wild turkeys compared with controls. Domesticated birds showed the greatest AFB_1-associated gene response with 3860 unique differentially expressed genes, whereas only 32 genes were found uniquely altered in the wild turkey. Indicative of epithelial response in the gut lumen to AFB_1, upregulation was observed for membrane proteins such as claudins (tight junction proteins) in both bird types. Statistically significant differences in gene expression were also observed between domesticated and wild birds within treatments. Among the genes showing differential expression in AFB_1-treated birds, significant enrichment was seen for membrane-associated and immunoglobulin-like domain containing genes. Among the Kegg gene pathways, calcium signaling was the most expressively represented. Results of this study identified genes and pathways differentially altered in aflatoxicosis and indicate differential responses of these genetically distinct birds.

Key Words: aflatoxin, turkey, intestine, cecal tonsil, RNAseq

544P DICER1 dysregulation and dsRNA accumulation in BCO chickens. E. Greene*[1], A. Al-Rubaye[1], S. Hennigan[2], J. Pleimann[3], D. Rhoads[1], R. Wideman[1], and S. Dridi[1], *[1]University of Arkansas, Fayetteville, AR, [2]Washington Regional Medical Center, Fayetteville, AR, [3]Ozark Orthopaedics, Fayetteville, AR.*

Bacterial chondronecrosis with osteomyelitis (BCO) is a considerable cause of lameness in broiler chickens, leading to production losses as well as increased welfare concerns in commercial flocks. It is associated with several bacterial species, with Staphylococcus being the most prevalent organism. However, there is little mechanistic understanding of the disease pathogenesis and progression. We hypothesized that the bacterial infection associated with BCO induces double stranded RNA (dsRNA) accumulation in bone cells via dysregulation of DICER1, a ribonuclease III enzyme. This dsRNA accumulation, in turn, activates the NLRP3 inflammasome, leading to IL-1β release, and subsequent negative impact on bone cell viability. To test this hypothesis, one day-old male boiler chicks were randomly divided into 2 body weight-matched groups. The control group was reared on clean shaved wood litters at 50 birds/pen and the BCO group was maintained on the wire floor model developed by Wideman group. Birds were provided a standard diet and water ad libitum, and the photoperiod was set for 23L:1D. To minimize distress, birds were walked daily from d 15 to the end of the experiment. On d 56, birds were humanely euthanized and necropsied to verify and score BCO lesions. Bone samples were snap frozen in liquid nitrogen and stored at −80°C until further analysis. Human osteoblast (hFOB) cells were grown in the appropriate medium and treated with *Staphylococcus aureus*, *S. agnetis*, or *S. hyicus* at an MOI of 50:1. After 24h, cells were collected for downstream analyses. Gene and protein expression from bone samples and cells were analyzed via real time quantitative PCR and Western blot, respectively. Data were analyzed by one-way ANOVA or Student's *t*-test, as appropriate. Significance was set at $\alpha = 0.05$. In BCO tibia, Dicer1 gene and protein expression were downregulated and members of the inflammasome cascade (NLRP3, caspase 1, IL-1β) were upregulated as compared with control ($P < 0.05$). When hFOB osteoblasts were infected with *S. aureus* or *S. agnetis*, viability was reduced ($P < 0.01$), and Dicer1 gene and protein expression were downregulated ($P < 0.05$). NLRP3 gene expression was upregulated with *S. agnetis*, and caspase1 and IL-1β genes were upregulated with both *S. aureus* and *S. agnetis* ($P < 0.05$). In addition, several dsRNA species (Birddawg in BCO and Alu in hFOB) were increased as compared with control ($P < 0.05$). In conclusion, we have shown that in both BCO chicken and *Staphylococcus*-infected hFOB osteoblasts, DICER1 dysregulation can lead to accumulation of dsRNA, activation of the inflammasome, and decreased cell viability.

Key Words: Dicer1, inflammasome, BCO, dsRNA, *Staphylococcus*

545P Intracerebroventricular administration of leptin induces hepatic autophagy in chickens through AMPK pathway. E. Greene*[1], J. Flees[1], W. Bottje[1], T. Ohkubo[2], H. Maier[3], M. Cline[4], and S. Dridi[1], *[1]University of Arkansas, Fayetteville, AR, [2]Ibaraki University, Ibaraki, Japan, [3]The Pirbright Institute, Woking, United Kingdom, [4]Virginia Tech, Blacksburg, VA.*

Direct crosstalk between leptin (the satiety hormone) and autophagy (the intracellular self-digestion process) has been shown in mammalian central nervous system and peripheral tissues. Such interaction is still unknown in avian (non-mammalian) species. We aimed in the present study to determine the effect of central administration of leptin on autophagy pathway in chicken liver. Five day post-hatch male Hubbard × Cobb 500 chicks were randomly divided into 2 body-weight matched groups and assigned to intracerebroventricular (ICV) injection with artificial cerebrospinal fluid (aCSF, control) or recombinant mammalian leptin (625 pmol, 10 μL, n = 10/group). Feed intake was recorded at 30, 60, and 180 min after injection. At the end of the experiment, liver tissues were harvested and stored at −80°C until use. In vitro studies were conducted using spontaneously immortalized chicken embryonic hepatocytes (Sim-CEL) or CHO cells overexpressing the long isoform of chicken leptin receptor and STAT3. Cells were treated with recombinant ovine leptin (100 ng/mL) alone or in combination with compound C (AMPK inhibitor, 20 μM). Untreated cells were used as controls. The expression of key markers of autophagy were determined by RT-qPCR, Western blot and immunofluorescence techniques. Leptin treatment significantly upregulated the expression of Atg3, Atg5, Atg7, Beclin1, and LC3B in chicken liver as well as in Sim-CEL and CHO cells. These changes were accompanied with a significant increase in the expression of chicken leptin receptor, STAT3, and AMPKα1/2. Compound C, the AMPK antagonist, prevented the ability of leptin to induce autophagy in Sim-CEL and CHO cells indicating that the effect of leptin was mediated through AMPK pathway.

Key Words: autophagy, leptin, liver, AMPK

546P Neuroendocrine regulation of avian hypothalamic autophagy by leptin. J. Flees*[1], E. Greene[1], W. Kuenzel[1], W. Bottje[1], T. Ohkubo[2], H. Maier[3], M. Cline[4], and S. Dridi[1], *[1]University of Arkansas, Fayetteville, AR, [2]Ibaraki University, Ibaraki, Japan, [3]The Pirbright Institute, Woking, United Kingdom, [4]Virginia Tech, Blacksburg, VA.*

The highly conserved intracellular self-eating process, autophagy, plays an integral role in maintaining cellular homeostasis. Although autophagy is extensively studied in mammals and yeast, there is still a paucity of information on autophagy regulation in avian species. The present study aimed, therefore, to determine the regulation of hypothalamic autophagy by leptin and define its underlying mechanisms in chickens. Five day post-hatch male Hubbard × Cobb 500 chicks were

randomly divided into 2 body-weight matched groups and assigned to intracerebroventricular (ICV) injection with artificial cerebrospinal fluid (control) or recombinant mammalian leptin (625 pmol, 10 μL, n = 10/group). Feed intake was recorded at 30, 60, and 180 min after injection. At the end of the experiment, hypothalamic tissues were harvested and stored at −80°C until use. Brain slices were obtained from 3 to 5 d-old chicks and hypothalamic organotypic cultures were conducted using cell inserts in presence of recombinant leptin (100 ng/mL) alone or in combination with compound C (AMPK inhibitor, 20 μM). Untreated cultures were used as a negative control. The expression of target genes and proteins were determined by RT-qPCR and Western blot, respectively. Data were analyzed by Student 't" test using the Graph Pad prism software. ICV-leptin administration reduced feed intake, upregulated the expression of feeding-related hypothalamic neuropeptides (NPY, CRH, orexin), and activated leptin receptor (Ob-Rb)-STAT3 and AMPK signaling pathway. The protein levels of autophagy-related markers (Atg3, Atg5, Atg7, Beclin, and LC3B) were significantly increased in the hypothalamus of leptin-treated chickens as well as in leptin-treated hypothalamic organotypic slices. Blocking AMPK activation attenuated leptin-induced autophagy in hypothalamic organotypic cultures. Together our data indicated that leptin induced autophagy in chicken hypothalamus through ObRb-STAT3-AMPK pathway.

Key Words: autophagy, leptin, hypothalamus, AMPK, chicken

547P Proteomics analysis of bacterial chondronecrosis with osteomyelitis (BCO) lesions in broiler tibia. E. Greene*[1], J. Cook-Turner[1], R. Liyanage[1], A. Al-Rubaye[1], S. Hennigan[2], J. Pleimann[3], D. Rhoads[1], R. Wideman[1], and S. Dridi[1], *[1]University of Arkansas, Fayetteville, AR, [2]Washington Regional Medical Center, Fayetteville, AR, [3]Ozark Orthopaedics, Fayetteville, AR.*

Bacterial chondronecrosis with osteomyelitis (BCO) is a significant cause of lameness, and leads to production losses as well as increased welfare concerns in broiler chickens. However, there is little mechanistic understanding of the disease pathogenesis and progression. Here, we used a proteomics approach to identify differentially expressed (DE) proteins between control and BCO chicken tibia. One day-old male boiler chicks were randomly divided into 2 body weight-matched groups. The control group was reared on clean shaved wood litters at 50 birds/pen (6 pens/group) and the BCO group was maintained on the wire floor model developed by Wideman's group. Birds were provided a standard diet and clean water *ad libitum*, and the photoperiod was set for 23L:1D. To minimize distress, birds were walked on a daily basis from d 15 to the end of the experiment (d 56). On d 56, birds were humanely euthanized and necropsied to verify and score BCO lesions. Bone samples were snap frozen in liquid nitrogen and stored at −80°C until further analysis. Proteins from healthy and BCO tibia were extracted and run on an SDS-PAGE gel, stained, then cut into sections. The sections were subject to in-gel trypsin digestion and sent for analysis by LC-MS/MS at the State Wide Mass Spectrometry Facility, University of Arkansas at Fayetteville. MASCOT and Scaffold analysis identified a total of 378 proteins. Quantitative analysis identified 51 differentially expressed proteins (*t*-test, $P < 0.05$). Of those, there were 37 up- and 14 downregulated proteins in BCO vs. normal tibia. To identify potential protein-protein interaction networks in BCO lesions, DE proteins were analyzed using STRING software. STRING identified 36 nodes with an average node degree of 3.15 (protein-protein interaction enrichment, $P = 0.001$). Data was log2 transformed and Ingenuity Pathway Analysis (IPA) identified Cell Death and Survival, Skeletal and Muscular Disorders, and Protein Folding as enriched disease and function categories. Enriched IPA canonical pathways included EIF2 signaling, Protein Kinase A signaling, Acute phase Response Signaling, Unfolded Protein Response, and Cavolar-Mediated Signaling. To validate and confirm the proteomic data, Western blot was performed for 2 proteins in BCO and control birds. HSP90 and OSTF1 protein was significantly upregulated (40% and 327%, respectively; $P < 0.05$) in the tibia of BCO compared with the control chickens. These data provide insight into the nature of BCO and will be useful in generating future mechanism-based approaches (nutrition and/or management) to prevent or reduce BCO incidence.

Key Words: BCO, proteomics

548P Histone methyltransferase inhibitors synergizes with butyrate in promoting host defense peptide gene expression in chickens. B. Chen*, W. Lyu, Q. Yang, and G. Zhang, *Oklahoma State University, Stillwater, OK.*

Host defense peptides (HDPs) have both antimicrobial and immunomodulatory properties. Dietary modulation of endogenous HDP synthesis is being explored as an alternative approach to antibiotics in disease control and prevention. Epigenetically, increased acetylation of histones in the HDP gene promotor is known to enhance the expression of many HDP genes. However, little is known about the impact of histone methylation on HDP gene expression. Following a high throughput screening, we identified several histone methyltransferase (HMT) inhibitors such as 3-Deazaneplanocin A, UNC0646, and UNC0638 with a strong ability to induce avian β-defensin 9 (*AvBD9*) gene expression. To confirm the efficacy of HMT inhibitors in HDP gene induction, chicken HTC macrophage cells were exposed to different doses of HMT inhibitors for up to 24 h, followed by total RNA isolation. The *AvBD9* mRNA expression level was evaluated by quantitative RT-PCR using the ΔΔCT method with glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as the reference gene. All 3 selected HMT inhibitors showed a clear and significant dose-dependent induction ($P < 0.05$) of *AvBD9* mRNA expression. To further examine a possible synergy between HMT inhibitors with butyrate, a short-chain fatty acid and a well-known histone deacetylase inhibitor, HTC cells were stimulated with 2 mM sodium butyrate in the presence or absence of different concentrations of HMT inhibitors for 24 h, followed by quantitative RT-PCR analysis of the *AvBD9* mRNA expression. Our results indicated that all 3 HMT inhibitors synergized strongly with butyrate in *AvBD9* induction. For example, the *AvBD9* mRNA expression level was at least 30-fold higher in response to the combination of UNC0638 and butyrate than either compound alone. In summary, we confirmed the HDP-inducing activity of 3 representative HMT inhibitors in chicken HTC macrophages. For the first time, we revealed that HMT inhibitors are synergistic with butyrate in HDP gene induction, suggesting that simultaneous demethylation and acetylation of the HDP gene promotors are cooperative in HDP gene transcription. Our results suggested the potential of using a combination of these compounds as alternatives to antibiotics in infectious disease control and prevention for poultry.

Key Words: host defense peptides, butyrate, histone methyltransferase inhibitors, histone deacetylase inhibitors, epigenetic regulation

550P Transcriptomic analysis of the shell gland in layers identifies novel genes in eggshell biomineralization. N. Sah*, D. Kuehu, V. Khadka, R. Jha, and B. Mishra, *University of Hawaii at Manoa, Honolulu, HI.*

Over 10% of the eggs produced in poultry farms are lost due to soft-eggshell breakage which accounts for a huge economic loss to the egg industry, thus needs to be addressed. The oviductal shell gland of a laying hen provides the biological environment for the eggshell mineralization. The objectives of this study were to 1) identify the novel differentially expressed genes (DEGs) and biological pathways in the shell gland (laying vs non-laying) involved in the eggshell formation, and 2) validate the identified novel genes in the laying, non-laying, and molter hens. Hy-line white hens (45–60 weeks), including 12 laying, 6 non-laying, and 6 molters hens were randomly housed in individual pens. Laying hens were euthanized at 2 different time points (3h, and 15–20h postovulation, p.o.) for the samplings of shell gland tissues. Non-laying and molter hens were euthanized randomly, and shell gland tissues were collected for RNA extraction. Total RNA from laying hens at 15–20h p.o. (n = 3) and non-laying (n = 3) hens were subjected to high-throughput sequencing. The mapped genes with FDR adjusted P-value < 0.05 and fold change > 2 were considered differentially expressed. In the laying hens at 15–20h p.o., 616 genes were identified, of which, 276 and 340 were up- and downregulated, respectively. Highly upregulated genes from the RNA-seq data having a putative role in calcium remodeling were further validated in all the experimental groups using real-time PCR. Among the upregulated genes, *Otopetrin 2* (modulating the influx of calcium) and *Calcitonin* (bone mineralization) had the highest expression ($P < 0.05$) in the shell gland of layers at 15–20h p.o. (around egg shell formation). The expression of *Plasma membrane* Ca^{2+} *transporting 2* (calcium transporter) was highest ($P < 0.05$) at 3h p.o. followed by 15–20h p.o. *Stanniocalcin 2* (biomineralization) had the highest expression level in layers at 15–20h p.o., an intermediate expression at 3h p.o. and molters, and lowest expression in non-layers. Kyoto Encyclopedia of Genes and Genomes pathway analysis showed that the upregulated genes belonged to the enriched calcium signaling pathway. Gene Ontology enrichment analysis revealed that the biological processes for sodium and potassium ion-transport were significantly enriched ($P < 0.05$) due to the upregulated genes. In conclusion, identified novel genes from the egg shell gland may increase the bioavailability, mineralization, and remodeling of calcium for the eggshell formation and can potentially be used to enhance the quality eggshell production traits in chickens, thereby reducing the loss.

Key Words: calcium remodeling, eggshell, layer, RNA sequencing, shell gland

551P Identification of regulators of the hepatic expression of let-7c and its target FASN in chickens. J. Hicks[2], T. Porter*[1], and H.-C. Liu[2], [1]*University of Maryland, College Park, MD,* [2]*North Carolina State University, Raleigh, NC.*

Hepatic fatty acid oxidation of yolk lipoproteins provides the main energy source for chick embryos. After hatching these yolk lipids are rapidly exhausted and metabolism switches to the carbohydrate-based energy in feed. We recently demonstrated that a large number of microRNAs (miRNAs) are key regulators of hepatic metabolic pathways during this metabolic switch. MiRNAs are small non-coding RNAs that post-transcriptionally regulate gene expression in most eukaryotes. We have previously shown that hepatic expression of the miRNA let-7c is much higher in the embryo and steadily declines post-hatching in both layers and broilers. Conversely, hepatic expression of its target gene FASN, substantially increases post-hatching. The present study was undertaken to further elucidate the molecular regulatory mechanisms of the metabolic switch in chickens by utilizing a yeast one-hybrid system to identify potential regulators of let-7c and FASN expression. Promoter regulatory elements for gallus gallus let-7c and FASN were identified using PROSCAN and cloned from Ross 708 genomic DNA. A Ross 708 hepatic cDNA library was created using the Y1H gold system (Clontech) for prey screening. For library construction, total RNA was purified from liver tissue and pooled from E18, E20, D0, D1 and D3 birds (n = 6). Approximately 2.2 million colonies and 2.5 million colonies were screened for let-7c and FASN, respectively. For let-7c, 2 regulatory factors were identified, FOXA2 and HDGF. FOXA2 has been shown to regulate the production of numerous liver proteins, including HNF4A and HNF6, among others. HDGF is a major regulator of hepatocyte proliferation and differentiation in mammals. For FASN, 4 potential regulatory proteins were found, HDAC2, CHREBP, PKNOX1 and SRF. HDAC2 is a member of the histone deactylase family and regulates many different cellular processes. CHREBP binds carbohydrate response elements to regulate several glycolytic and lipogenic genes. In mice, PKNOX1 has been shown to regulate hepatic lipogenesis. In mammals SRF alters the expression of a variety of pathways in response to changes in blood lipid and glucose levels. Taken together, a complex regulatory cascade for hepatic metabolic processes in chickens is suggested involving let-7c and the transcription factors identified in the present study.

Key Words: miRNA, metabolism, liver, gene regulation, development

552P Effects of heat stress on hypothalamic and pituitary mRNA expression in broilers. R. Beckford*[1], L. Farley[1], L. Ellestad[2], M. Proszkowiec-Weglarz[3], K. Brady[1], and T. Porter[1], [1]*University of Maryland, College Park, MD,* [2]*University of Georgia, Athens, Georgia,* [3]*USDA, ARS, NEA, Beltsville, MD.*

Heat stress in chickens occurs when the ambient temperature rises above the thermoneutral temperature and birds are unable to efficiently reduce their body heat. Effects of heat stress on broiler production include increased mortality rate in the flock and decreased performance by the birds that survive. Identifying a management strategy that reduces the negative consequences of heat stress and that is not labor intensive or costly would be beneficial. To effectively evaluate these strategies, it is important to measure not only production parameters but also the underlying physiological mechanisms. The hypothalamus and pituitary regulate metabolism and stress responses. Therefore, the objective of this study was to evaluate corticotropic and thyrotropic mRNA expression in the hypothalamus and pituitary of broiler chickens subjected to heat stress. At 24 days of age, Ross 708 male chickens were placed into battery cages in 8 grower batteries in each of 2 rooms maintained at 22°C (10 birds/cage, 2 cages/battery). On d 31, four batteries from each room were moved into two rooms with an elevated ambient temperature (35°C) for 8h for the heat stress challenge (HS). Remaining batteries stayed in the two thermoneutral rooms with an ambient temperature of 22°C (no heat stress; NHS). After the 8h heat stress challenge, birds were euthanized and hypothalamus and pituitary samples collected from 16 birds per treatment (2 birds/cage/battery/room), flash frozen and stored at −80°C until RNA extraction. Reverse transcription qPCR was used to compare mRNA levels between HS and NHS birds. Differences in

expression were determined using mixed model ANOVA (SAS v9.4, Cary NC) to compare mRNA levels of each target gene normalized to PGK1 and GAPDH (n=16/treatment) mRNA levels in the pituitary and hypothalamus respectively. In the adrenocorticotropic axis, expression of pituitary corticotropin-releasing hormone receptor 1 was significantly downregulated ($P < 0.001$) while corticotropin-releasing hormone receptor 2 was 2-fold higher ($P = 0.001$) in the HS birds. Interestingly, pituitary mRNA levels for proopiomelanocortin were not significantly ($P > 0.05$) different. In the thyrotropic axis, heat stress increased the expression of metabolic related genes. Both thyroid hormone receptor β ($P = 0.01$) (2.8-fold) and thyroid stimulating hormone β ($P = 0.009$) (1.4-fold) were higher in HS than NHS birds. Heat stressed birds respond with a myriad of molecular events, and this study provides insight into the events occurring within the hypothalamus and pituitary. Understanding these pathways and their regulation during heat stress will enable researchers to better evaluate management strategies to combat heat stress.

Key Words: broiler, heat stress challenge, hypothalamus, pituitary, management strategies

553P Differential pituitary response to GNRH and GNIH in low and high egg producing turkey hens. K. Brady*[1], J. Long[2], and T. Porter[1], *[1]University of Maryland, College Park, MD, [2]USDA, Beltsville, MD.*

Turkey hens with low egg production have a negative economic impact on the industry as these hens produce fewer poults yet cost the same to maintain as their high egg-producing counterparts. Egg production begins with the secretion of gonadotropin releasing hormone (GNRH) from the hypothalamus causing luteinizing hormone (LH) to be released from the pituitary. LH plays a role in triggering the ovulation of follicles from the ovary and thus is critical for egg formation in the hen's reproductive tract. LH also is negatively regulated by gonadotropin inhibitory hormone (GNIH) secretion from the hypothalamus. To determine if low egg-producing hens (LEPH) and high egg-producing hens (HEPH) respond differently to GNRH and GNIH, pituitaries from LEPH (n = 3) and HEPH (n = 3) were recovered post mortem, dissociated for cell culture, and treated with GNRH or GNIH at concentrations of 0, 10^{-9}, 10^{-8}, or 10^{-7} M for 6 or 24 h. After incubation for 6 or 24 h, cells were recovered from each treatment for RNA isolation. The mRNA levels of key receptors and the main targets of GNRH and GNIH action were assessed through reverse transcription-quantitative PCR. A 2-way ANOVA, using the mixed models procedure of SAS, compared the normalized mRNA levels of pituitary cells from LEPH and HEPH among the treatments for each time point. In response to GNRH treatment for 6 h, cells from HEPH showed reduced gonadotropin inhibitory hormone receptor (GNIHR) expression relative to cells from LEPH ($P < 0.05$). Pituitary cells from HEPH also upregulated the β subunit of luteinizing hormone (LHB) mRNA after GNRH treatment for 6 and 24 h ($P < 0.05$). In response to GNIH treatment, cells from HEPH showed increased gonadotropin releasing hormone receptor (GNRHR) expression after 6 h relative to cells from LEPH ($P < 0.05$). Interestingly, pituitary cells from HEPH had increased LHB expression after 6 and 24 h of GNIH treatment ($P < 0.05$). Cells from HEPH exhibited lower expression of GNIHR compared with cells from LEPH at basal conditions for each hormone and time point ($P < 0.05$). At the transcript level, pituitary cells from LEPH and HEPH responded differently to GNRH and GNIH. Cells from HEPH upregulated genes that were associated with ovulation stimulation, whereas cells from LEPH upregulated genes that were associated with inhibition of ovulation. Differential pituitary cell response to GNRH and GNIH between LEPH and HEPH may play a role in the variation of egg production that occurs in the turkey industry.

Key Words: turkey, egg production, pituitary, gene expression, cell culture

554P Potential miRNA regulators of differential HPG axis gene expression between low egg producing and high egg producing turkey hens. K. Brady*[2], H.-C. Liu[1], J. Hicks[1], J. Long[3], and T. Porter[2], *[1]North Carolina State University, Raleigh, NC, [2]University of Maryland, College Park, MD, [3]USDA, Beltsville, MD.*

Expression differences exist in key genes of the hypothalamo-pituitary-gonadal (HPG) axis in low egg producing hens (LEPH) and high egg producing hens (HEPH); however, regulation of these differences is unknown. MicroRNAs (miRNAs) are small non-coding RNAs that play a role in post-transcriptional regulation by blocking translation or targeting mRNA degradation, causing lower levels of the target protein. MicroRNAs, potentially involved in the regulation of the HPG axis, were assessed in LEPH and HEPH. MiRanda, a miRNA target prediction algorithm, was used to scan HPG axis genes that were differentially expressed between LEPH and HEPH for possible miRNA binding sites. The hypothalamus, pituitary, and the granulosa layers of the largest follicle (F1G) and fifth largest follicle (F5G) were sampled from LEPH and HEPH (n = 3/group). Expression of miRNAs with predicted binding sites was examined in each tissue by reverse transcription-quantitative PCR. Data were analyzed with a 2-way ANOVA using the mixed models procedure of SAS. Potential regulatory miRNA were identified first by significant expression differences in LEPH and HEPH and second by an inverse relationship with levels of their predicted mRNA target ($P < 0.05$). In the hypothalamus, miR-7464–3p, miR-222a, and miR-222b-3p were increased in HEPH, in comparison to LEPH, and were identified as possible regulators for the lower gonadotropin inhibitory hormone (GNIH) levels seen in HEPH in contrast with LEPH ($P < 0.05$). Also, miR-1794 and miR-222a, with predicted progesterone receptor (PGR) binding, were upregulated in HEPH, while PGR was downregulated in HEPH ($P < 0.05$). In the pituitary, miR-1690–5p, miR-1658–3p, miR-1644, and miR-1587 were predicted to bind gonadotropin inhibitory hormone receptor (GNIHR) mRNA and showed higher expression in HEPH, inversely correlating with lower expression of GNIHR in HEPH ($P < 0.05$). In the F1G, predicted miRNA expression between HEPH and LEPH did not relate with previous mRNA expression differences ($P > 0.05$). In the F5G, miRNAs predicted to bind steroidogenic acute regulatory protein (STAR) and cholesterol side chain cleavage enzyme (CYP11A1), miR-1466 and miR-1658–3p, showed increased expression in HEPH, coupled with decreased expression of STAR and CYP11A1 in HEPH ($P < 0.05$). Moreover, expression of miR-6688–3p, predicted to bind luteinizing hormone receptor (LHCGR), was upregulated in HEPH, compared with downregulation of LHCGR in HEPH ($P < 0.05$). Validation of candidate regulatory miRNA by luciferase reporter assays is vital to further understand the input of the identified miRNAs to differential HPG axis gene expression seen in LEPH and HEPH.

Key Words: turkey, egg production, HPG axis, miRNA, gene expression

555P Effect of vitrification-warming procedures on testis from young chicks. C. C. Quach*[1], C. Lessard[2], J. Benson[1], C. Auckland[2], and P. Hind[2], *[1]University of Saskatchewan, Saskatoon, SK, Canada, [2]Agriculture and Agri-Food Canada, Saskatoon, SK, Canada.*

Vitrification can be used to preserve gonadal tissue from 1 to 3 d old chicks. Vitrified-warmed gonads can be transferred into recipients of same age to sustain their growth and maturation. The goal of this study was to determine damage to testes following vitrification and warming procedures. Gonads were harvested from newly hatched chicks and

vitrified following these steps: 1) 15 min in VS1 (7.5% Ethylene Glycol and 7.5% DMSO); 2) 3 min in VS2 (15% Ethylene Glycol, 15% DMSO, and 0.5M sucrose); 3) plunged in liquid nitrogen. After a minimum of one week of storage, vitrified tissues were warmed at room temperature into dishes containing 1 M of sucrose for 5 min, 0.5 M of sucrose for 5 min, 0.25 M of Sucrose for 5 min, and then holding medium (DPBS with 20% FBS) for a minimum of 15 min. Vitrified-warmed and fresh testes were fixed with Bouin's solution and embedded in paraffin. Sections of 4 μm of thickness were cut and stained with hematoxylin-PAS. Tissue integrity was subjectively evaluated using an adapted scoring system developed by Milazzo et al. (2010, Journal of Andrology 31, 617–630). A nonparametric test (Mann-Whitney U test) was performed to compare damage between fresh and vitrified tissues. Our study revealed that vitrification-warming procedures significantly affect the integrity of the testis cells: cellular distinctions between Sertoli cells and germ cells were difficult to visualize; an increased number of observations of pyknotic nuclei and detachment of intratubular cells from the myoid cells were noticed; and gaps or shrinkage of cells were observed ($P < 0.001$). Interestingly, this damage does not appear to impede in the ability of the testis to grow and reach maturity: full spermatogenesis was observed on matured recipients having vitrified-warmed testis. Note: sperm recovered from these matured testis grafts was not evaluated for their ability to fertilize an egg yet. In summary, vitrification-warming procedures will affect the cellular integrity of the chicken testis, suggesting the need to optimize this preservation technique for long-term storage.

Key Words: vitrification-warming procedures

556P Ontogenic changes in the Cobb broiler following early-embryonic induction of growth hormone. B. Sparling*[1], J. Payne[1], L. Vaccaro[1], T. Porter[2], and L. Ellestad[1], *[1]University of Georgia, Athens, GA, [2]University of Maryland, College Park, MD.*

We have previously reported that in ovo injection of corticosterone (CORT) during mid-embryonic development and in vitro treatment of cultured embryonic day (e) 11 pituitary cells results in earlier growth hormone (GH) induction in chickens. However, long-term effects of this treatment, particularly on embryonic and post-hatch growth and metabolism, are not well understood. In a study conducted over 20 years ago with Shaver broilers, CORT treatment on e11 increased body weight after hatch in a sex-dependent fashion, with CORT-treated males weighing 80 g more than controls at d 42, due in part to a significant increase in breast muscle mass. However, substantial improvements in growth performance have occurred through commercial genetic selection since that time. Therefore, our objective was to grow modern broiler chickens from CORT-treated eggs to market age to further test the hypothesis that early induction of GH can increase performance parameters by measurement of body weight gain, feed efficiency, carcass characteristics, and gene expression. Cobb 500 eggs were left uninjected or injected with saline or CORT (200 ng/egg) on e11. Anterior pituitaries, liver, and muscle tissue were collected 6h and 12h after injection, and on e13, e16, and e19 ($n \geq 4$ per tissue per age) for evaluation of gene expression within the somatotropic axis. On day of hatch, chicks were sexed, weighed, and assigned to pens by treatment (9 females and 9 males/pen; n = 9 pens per treatment). Birds were reared through d 42, and body weights and feed intake were recorded every 2 weeks. Data were analyzed by 3-way ANOVA followed by means comparisons using Tukey's HSD test. Injection with CORT significantly increased levels of GH mRNA in the pituitary and GH-receptor mRNA in the liver relative control treatments at 6 h ($P < 0.05$). Effects of treatment on body weight, feed intake, body weight gain, average daily gain, and feed conversion ratio throughout the grow out period were statistically similar ($P > 0.05$). Though, collective meat weights (pectoralis major and minor, thigh, drumstick, and wing) as a percentage of cold empty carcass weight tended to be higher for CORT-treated birds than controls (~1.0%, $P = 0.0815$). CORT-treated birds, irrespective of sex, tended to have higher pectoralis major (0.5–0.7%, $P = 0.1364$) and drumstick (0.07–0.14%, $P = 0.4301$), as a percentage of empty carcass weight. Although we did not observe any differences between treatments on body weight gain or feed efficiency, the tendency for birds from CORT-injected eggs to have increased overall meat yield indicates that early induction of GH may have a lasting effect on nutrient partitioning within the birds, ultimately leading to increased muscle gain.

Key Words: growth hormone, corticosterone, mRNA abundance, embryo, growth

557P The impact of age and selection on intestinal morphology at 14 and 42 days of age in broilers and layers. T. Bong*[1], L. Reber[2], S. Orlowski[2], N. Anthony[2], S. Rochell[2], and D. Koltes[1], *[1]Iowa State University, Ames, IA, [2]University of Arkansas, Fayetteville, AR.*

Selection for production traits over the past 60 years has led to significant changes in production, specifically meat and egg yields. However, selection for a single trait will often influence other traits. Therefore, we wanted to determine if divergent selection of broilers and layers impacted the morphology of the intestine. To determine potential differences with selection, 3 lines of chicken (Leghorn, 1995 and 2015 random bred) were reared in floor pens and provided a standard broiler starter and grower diet. Eight birds were weighed then euthanized at d 14 and 42 to determine changes in intestinal length, villus height, and luminal circumference. Each parameter was analyzed by intestinal section using the PROC Glimmix procedure in SAS with sex, age, line, and the interaction of line by age fit as fixed effects. Pen was fit as a random effect. Body weight was different across line, age, and line by age interaction with layers growing at a slower rate compared with broilers ($P < 0.01$). Intestinal section length, villus height, and luminal circumference are different across lines, regardless of the intestinal section. As expected, measurements were larger in the 2 broiler lines compared with the layer line ($P < 0.05$). Intestinal length and luminal diameter increased with age across all 3 intestinal sections ($P < 0.05$). Villus height increased in the jejunum and ileum with age ($P < 0.05$) but was similar at d 14 and 42 in the duodenum ($P < 0.10$). An age by line interaction was only observed for the ileal length in the study. In conclusion, the lack of continued growth of villi in the duodenum from d 14 to 42 may suggest early maturation of the duodenal section, regardless of line. However, it is unclear how this may alter mineral absorption or enteric signaling. Therefore, additional research is warranted to determine if cell composition of the villus is altered with age or line, and if this could be impacting traits associated with animal well-being in both the broiler and layer industries.

Key Words: villus height, luminal circumference, nutrient absorption, crypt depth

558P Effect of the use of carvacrol and cinnamaldehyde in replacement of performance improving additives in meat quality of broilers. C. Facchi*, G. Bosetti, E. Aniecevski, F. Leite, P. Guarnieri, G. Rossato, L. Griebler, and T. Petrolli, *UNOESC, Xanxerê, Brazil.*

The use of antibiotic growth promoters in broiler feedinghas been increasingly restricted, requiring the development of new molecules to adequately replace this class of feed additives in bird diets. Herbal extracts are a promising alternative, however, their influence on the quality of the meat has not been elucidated yet. The objective of this study was to evaluate the influence of the addition of carvacrol and cinnamaldehyde in broilers feeding on cooking losses, color, water retention capacity, shear force and lipid oxidation of meat. A completely randomized experimental design was done, being composed by 5 treatments and 8 replications, in which: T1 - negative control; T2 - positive control (Virginiamycin 30 ppm); T3 - 100 ppm essential oils; T4 - 200 ppm essential oils; T5 - 400 ppm essential oils. At 40 d of age, one bird per experimental unit was slaughtered, and the left fillet of the birds' breast was collected. The results were analyzed by ANOVA and means were compared by SNK test, at 0.05 of significance.No differences on cooking losses ($P = 0.67$), luminosity ($P = 0.17$), a* pigment($P = 0.66$), b* pigment ($P < 0.05$), water retention capacity ($P = 0.21$), shear force ($P = 0.12$) and lipid oxidation ($P = 0.44$), indicating that carvacrol and cinnamaldehyde can be used adequately in broiler feeding, without compromising the quality of meat, when used in substitution of antibiotics as growth promoters in the broiler diet.

Key Words: essential oils, growth promoter, poultry nutrition, shelf life, sensory analysis

559P Ameliorative effects of branched-chain amino acid-rich chicken liver hydrolysates on the high fat diet-induced metabolic syndrome. Y.-H. Wu*[1], Y.-L. Lin[1], J.-W. Chen[2], J.-K. Tseng[3], C.-H. Chou[1], and Y.-C. Chen[1], *[1]National Taiwan University, Taipei City, Taiwan, [2]Council of Agriculture, Executive Yuan, Taipei, Taiwan, [3]Asia University, Taichung, Taiwan.*

The broiler liver is regarded as worthless and cheap stuff due to restricted usages. However, it contains plenty of nutrients such as amino acids, and many reports had revealed the bioactivity, i.e., anti-obesity and hypolipidemic effect, of food protein hydrolysates. Thus the development of functional ingredients may be practical and potential. In the other hands, metabolic syndrome (MtS), clustered of few medical diagnoses (central obesity, decreased high-density lipoprotein cholesterol, hyperlipidemia, hypertension, fatty liver, and prediabetes), is the crucial issue to both human beings and their animal partners. Recently, the Taiwan poultry industry tends to follow the global trend of antimicrobial growth promoter (AGP) forbidden, but the price hike due to cost considerations may impact to the consumer; therefore, development of functional ingredients from by-product may benefit the Taiwan poultry industry. In this study, the pieces of evidence indicated that chicken liver hydrolysates (CLHs) could alleviate the MtS in vitro and in vivo.It had already found that pepsin-digested CLHs enhance anti-oxidative and perform anti-fibrotic effect in a different mouse model in vivo. The liver tissue was hydrolyzed by pepsin (ES ratio=1:400) under pH2 two hours, and the hydrolysate liquor was neutralized to pH7. Besides, the hepatic lipid would be removed during centrifugation and filtration at 4C. This study was divided into two stages. First, the oleic acid-induced FL83B cell model was conducted to investigate the attenuative effect of intracellular lipid accumulation in vitro. Second, the effectiveness was reproduced via high fat-induced mice model in vivo. The 24 male C57/BL6 mice were randomly divided as follows: Control, HFD (46.5% of caloric is from fat), and HFD+CLH (170 mg/kg) were mixed with feed according to their feed intakes and body weights. The one-way ANOVA was conducted to all categorical data via SAS program, and the significance level was set as 0.05. As results, CLHs supplementation decreased ($P < 0.05$) body weights, peri-renal fat mass, abdominal fat mass, and serum TC in HFD-fed mice. It also improved ($P < 0.05$) the atherosclerosis index which was calculated by values of LDL-C and HDLC. Besides, it reduced ($P < 0.05$) serum and liver TBARS, elevated ($P < 0.05$) serum TEAC and anti-oxidative capacities as well as liver anti-oxidative capabilities. According to results of a pathohistological analysis, adipocyte sizes of peri-renal fat tissue were significantly decreased ($P < 0.05$) in those CLH-fed mice. Moreover, it attenuated chronic injury of liver, kidney, and heart under CLH supplementation via Sirius red stain. In conclusion, CLHs supplementation could attenuate HFD-induced MtS.

Key Words: chicken liver hydrolysates, pepsin, metabolic syndrome, FL83B cell, high-fat diet

560P Relationship between the woody breast and white striping myopathies in broiler breast meat. B. Bowker*, H. Zhuang, and S.-C. Yoon, *USDA-ARS, Athens, GA.*

The woody breast (WB) and white striping (WS) myopathies that occur in the *Pectoralis major* muscles of broilers are associated with the fast growth rate and large size of modern broilers. These myopathies often occur in the same breast fillet. The 2 myopathies have similar effects on the histology and composition of the muscle tissue. However, the relationship between these 2 myopathies is not well defined. The objective of this study was to evaluate the relationship of the occurrence and degree of severity between the WB and WS myopathies in broiler breast fillets. Over 2800 breast fillets were collected from the deboning line of a commercial processing plant that slaughters large broilers (3.6–4.1 kg live weight). Individual breast fillets were weighed and assessed independent WB and WS scores from 1.0 to 3.0 in 0.5 score increments (normal = 1; moderate = 2; severe = 3). Data were analyzed using chi-squared, frequency, and correlation analysis. Chi-squared analysis indicated that there was a significant relationship between the WB and WS conditions ($P < 0.0001$). Approximately 94% of all WB fillets (WB score $\geq$1.5) also exhibited WS. Of the fillets that did not exhibit WB, 54% exhibited WS. Of the fillets that exhibited severe WB, 28% had severe WS, 51% had moderate WS, and 21% had mild or no WS. Approximately 83% of all WS fillets (WS score $\geq$1.5) exhibited WB. Of the fillets that did not exhibit WS, 26% exhibited WB. Of the fillets that exhibited severe WS, 41% had severe WB, 46% had moderate WB, and 13% had mild or no WB. Overall, there was a significant positive correlation between WS and WB scores ($r = 0.55$, $P < 0.0001$), but the incidence of the myopathies varied by fillet weight which was moderately correlated to WB ($r = 0.44$, $P < 0.0001$) and WS ($r = 0.32$, $P < 0.0001$) scores. Overall, WS occurred in 83% and WB occurred in 73% of the fillets. For fillets less than 407 g (lowest quartile range), 27% of fillets had moderate or severe WS and 22% of fillets had moderate or severe WB. However, for fillets >528 g (highest quartile range), 70% of fillets had moderate or severe WS and 77% of fillets had moderate or severe WB. The strength of the relationship between WS and WB scores changed with fillet weight ($r = 0.63$ for fillets <407 g; $r = 0.51$ for fillets 407–469 g; $r = 0.43$ for fillets

469–528 g; r = 0.39 for fillets >528 g). Data suggest that although the occurrence of the WS and WB myopathies in breast fillets are closely related, breast fillets exhibiting WS without WB are more likely to be observed than WB fillets without WS. Furthermore, data suggest that the strength of the relationship between the WB and WS myopathies in breast meat from large broilers is influenced by fillet weight.

Key Words: broiler, breast meat, myopathy, white striping, woody breast

561P Time course of chemical and functional property changes in egg yolk from laying hens fed docosahexaenoic acid-rich microalgae. B. Liu[2], J. Jiang[2], X. Yan[3], G. Lin[3], D. Yu[4], R. Delles*[1], and Y. Xiong[5], *[1]Alltech, Nicholasville, KY, [2]Jiangnan University, Wuxi, Jiangsu, China, [3]Alltech China, Beijing, China, [4]Zhejiang University, Hangzhou, Zhejiang, China, [5]University of Kentucky, Lexington, KY.*

This study evaluated dynamic changes in n-3 fatty acids, color, functional properties, and lipid oxidative stability of egg yolk from hens fed docosahexaenoic acid (DHA)-rich microalgae (*Aurantiochytrium* sp.) during a 56-d feeding period. A total of 180 laying hens (Hy-Line W-36, 26 wk old) were randomly assigned into 3 dietary treatments with 4 replicates of 15 birds in each: a corn-soybean meal standard diet (control) or diets supplemented with 0.5 or 1.0% microalgae (Alltech Inc. Nicholasville, Kentucky). The experimental diets were formulated to be isocaloric and isonitrogenous. The DHA content, yolk color, viscosity, and emulsification properties of egg yolk were measured on sampling days during feeding. Egg quality parameters and lipid oxidation (TBARS) were measured after 0, 14, and 28 d of refrigerated storage. Data were subjected to ANOVA using the General Linear Model procedure of SPSS 20.0 software. DHA concentration in whole eggs from hens fed 0.5 or 1.0% microalgae increased quadratically with feeding time with a plateau at about 30 d ($P < 0.05$). Afterward, the DHA content leveled off to constant values (172.7 ± 8.9 vs. 210.6 ± 7.4 mg/100 g egg) with the final n-6/n-3 ratio ranging from 3:1 to 5:1. In contrast, the DHA content of control eggs remained constant (70.07 ± 4.10 mg/100 g egg) throughout the feeding trial. Due to the microalgal carotenoids incorporated into egg yolk, the L^* value of yolk from hens fed microalgae decreased whereas a^* value increased ($P < 0.05$), corresponding to yolk Roche color scores. There were no significant changes in egg yolk functional properties (e.g., viscosity and emulsifying activity) related to DHA enrichment ($P > 0.05$). Lipid oxidative stability and quality characteristics of eggs during refrigerated storage were not affected by microalgae supplementation ($P > 0.05$). Results indicate that dietary microalgal DHA incorporation into eggs was efficient, and the feeding strategy had a minimal impact on yolk physical and functional properties or oxidative stability of shell eggs during subsequent refrigerated storage.

Key Words: microalgae, DHA, egg yolk, functional properties, oxidative stability

562P Improvement of chilling efficiency and product quality of broiler carcasses using sub-zero saline solutions. S. Viliani*, M. Metheny, H. C. Lee, D. Bennett, S. Hurley, and I. Kang, *Cal Poly, San Luis Obispo, CA.*

Carcass chilling in sub-zero saline solution has the potential to improve chilling efficiency and meat quality. The purpose of this research was to investigate the effect of sub-zero saline chilling on chilling efficiency and product quality of broiler carcasses. In Experiment I and II, eviscerated broiler carcasses were chilled using 3 (0% NaCl/0.5°C, 4% NaCl/-2.41°C, and 8% NaCl/-5.08°C) and 4 (0% NaCl/0.5°C, 1% NaCl/-0.6°C, 2% NaCl/-1.2°C, and 3% NaCl/-1.8°C) saline solutions, respectively. Data in 3 replications were evaluated by one-way ANOVA and a completely randomized design. A post-hoc analysis was performed using Duncan`s multiple range test to evaluate difference among treatments at $P < 0.05$. In Experiment I, breast fillets of broilers chilled in saline solutions showed lower shear forces than 0% NaCl control, regardless of salt content and temperature level ($P < 0.05$). In Experiment II, 3% NaCl/-1.8°C solution reduced the broiler chilling time by 22% over the water control, with an intermediate reduction (13 ~17%) observed for 1% NaCl/-0.6°C and 2% NaCl/-1.2°C solutions. The shear force was stepwise reduced as NaCl increased from 0 to 3% and solution temperature decreased from 0.5 to −1.8°C. However, no significant difference was observed for carcass chilling yield as well as salt content, cooking yield, pH, and R-value in breast fillets, regardless of chilling method. Based on these results, chilling of boiler carcass in 3% NaCl/-1.8°C or 4% NaCl/-2.41°C appears to be ideal for the improvement of chilling efficiency and meat tenderness. More research is required to elucidate any fundamental mechanism for the meat tenderness as well as for the potential in product safety improvement and overall processing cost reduction.

Key Words: sub-zero saline chilling, chilling efficiency, meat tenderness, broiler carcass quality

WPSA Lecture: The Current State and Future Demands of the Poultry Industry: Will We Meet Our Commitments?

563S The current state and future demands of the poultry industry: Will we meet our commitments? R. Kleyn*, *SPESFEED (Pty) Ltd., Broederstroom, South Africa.*

The primary objective of agribusiness is to ensure food security for the global population. It is estimated that increases in population size, coupled with socioeconomic changes such as urbanisation, demographics and increasing levels of affluence, will lead to a doubling of the demand for poultry products by 2050. This will need to occur in the face of climate change. A concurrent development is the demand by consumers that all food products be produced in a natural and sustainable manner. It is probable that the poultry industry possesses the technology, skills and capital to meet the expected demand targets. The questions which need to be addressed though are these; Will we be able to meet the demand for poultry products in a sustainable manner from environmental, social and financial standpoints? How will climate change impact the poultry industry? To what extent we will be prevented from using the technologies required to achieve these goals by public opinion? These aspects will be discussed in what is hopefully an objective and constructive manner.

Key Words: sustainability, climate change, consumer demand, technology, population growth

Informal Nutrition Symposium: Maintaining Production Efficiency in the Face of Changing Consumer Demands

564S Addressing consumer pressure: Diversifying production systems. R. Mitchell*, *Perdue Foods LLC, Salisbury, MD.*

Conventional production systems have the advantage of utilizing all legal means to improve production efficiency. Over the last several decades these systems have realized tremendous improvements in efficiency that have resulted in cost reductions to the consumer. This improvement has not come without controversy and many consumer groups have been highly critical of some practices of modern poultry production. Alternative production practices and niche markets have been developed to appeal to consumers concerned of specific practices. Alternative broiler production systems including organic, no antibiotics ever, free range, non-GMO and slow-growth among others will be discussed.

Key Words: alternative production system, niche markets, consumer, organic, informal nutrition symposia

565S Biological implications of slow broiler growth programs. M. Zuidhof* and D. Korver, *University of Alberta, Edmonton, AB, Canada.*

Increasingly, consumers are appealing for broiler meat products they perceive to be more wholesome or socially responsible. Such demands include product traits like appearance, taste, texture and nutritional content; animal welfare considerations such as skeletal and metabolic health and access to the outdoors or natural light; and assurance of food safety, including no antibiotics, clean production facilities, or even free of added hormones or genetic modifications. The latter examples demonstrate a clear lack of consumer knowledge about how food is currently produced. Slow growing broilers have become a quixotic focal point for their concerns. Much effort has been made to evaluate the agrobiology of slow growing traditional or heritage lines of chicken. Breeding specifically for efficient growth and higher yield traits in slow-growing chickens provides a clear advantage over using heritage lines. These breeding efforts will almost certainly provide the new normal to meet the demands of this market niche. The move toward slow-growing broilers will have many implications for welfare, product yield and quality, the environment and the ultimate cost of the final product. Careful evaluation of production realities reveals many unintended consequences. Land, feed and water usage will increase markedly with any move to slower growing broilers. Conservation of these resources is often a key consideration that is ignored by a romantic preference for slower growing broilers. However, slow-growing broilers may achieve some of the desired outcomes. Although selection for skeletal integrity and metabolic health has dramatically improved broiler health and welfare, biological limits to mature body weights, maturation rates, and likely meat quality are approaching. New research suggests that broiler breeder feed restriction will need to be relaxed in the near future to deal with an erosion of whole body fatness over decades. Though it not likely to be implemented only to reduce hunger, greater feed allowances will become mandatory to ensure sufficient body composition thresholds are met to initiate and sustain egg and chick production. This particular challenge would likely be less prevalent in slow growing broilers. Use of slow growing broilers will affect more variables than many consumers naively expect. Difficult tradeoffs between slow growth rate and animal welfare, the environment, and cost of production present a challenging optimization problem. There is no way to maximize all of the desired outcomes, and therefore it is important for all involved in this venture to embark with eyes wide open.

Key Words: slow growing broiler, chicken meat, market preferences, consumer demand, sustainability

566S Metabolic disorders associated with high production. H. Classen*, *University of Saskatchewan, Saskatoon, SK, Canada.*

Chickens used for meat production have been selected for decades for improved performance and it can be easily argued that broilers have been selected more extensively than any other livestock species. As a consequence, the improvement in performance associated with this selection has been outstanding with important consequences for feeding the world's population and the sustainability of chicken meat production in an environmentally challenged world. However, being the most selected animal also can lead to challenges, particularly unforeseen changes in other attributes, including those affecting bird well-being. Selection for extremes in any characteristic puts pressure on other bodily systems to support the advancement of the selected trait. Among these characteristics are metabolic disorders, which are non-infectious in nature, but result in significant pathological change or potentially death. Metabolic disorders that have received the most attention relate to the cardiovascular (sudden death syndrome, ascites) and musculoskeletal systems, but others exist or will in the future. For many of the specific conditions associated with these systems, more sophisticated selection techniques have minimized their importance in commercial production. However, how metabolic disease is measured and defined varies, and as a consequence so does the acceptable level of metabolic disease. Society will undoubtedly help establish acceptability levels and hopefully balance high performance benefits against real problems. So the question of whether high production is compatible with a low incidence of metabolic disorders is without a firm answer and will indeed need reevaluation with time. A saving grace is the power of genetic selection and as a consequence rapid changes that can occur to rectify existing or new issues.

Key Words: sudden death syndrome, ascites, leg disorders

567S Understanding how poultry's opponents play the message game. W. Jamison*, *Palm Beach Atlantic University, West Palm Beach, FL.*

Contemporary consumers process information regarding the poultry industry and poultry products using a variety of techniques and mental short-cuts which rarely include science or purely fact-based information. Instead, they understand information in terms of narrative frameworks and confirmation biases that are constructed largely independent of industry input or agricultural/academic information. Thus, it becomes very important for the poultry industry to communicate facts and information in terms and ways which consumers understand. Basically, messages must be highly salient, resonant, and valid to be persuasive.

Key Words: fact-based information, communication, consumers, poultry industry, poultry products

568S Maintaining broiler and breeder production efficiency in the face of changing consumer demands. A. Corzo* and S. Avendano, *Aviagen Inc., Huntsville, AL.*

Recent focus on sustainability of livestock production in both scientific literature and political agendas worldwide has been seen. Concomitantly, poultry meat is expected to have a primary role on satisfying the increasing demand for meat with a share of 44% to total meat production growth. Low production costs and affordability when compared with other meats have been attributed to making poultry the meat of choice in the developing world. Studies have shown that poultry production systems have a lower environmental impact when compared with other meat livestock productions. Aviagen currently offers efficient and high production broiler crosses such as the Ross 308 and 708. To satisfy market segments requesting poultry meat obtained from slower growth crosses, the company possesses a range of genotypes in its portfolio. All genotypes will have differing feed, water, and spatial requirements, being the Ross 308 and 708 genotypes those in our portfolio with the highest efficiency when utilizing these resources. Calculation of Greenhouse emissions, eutrophication potential, acidification potential and primary energy use suggest that the Ross 308 broiler, followed closely by the 708, are the most sustainable and least environmentally impactful of all Aviagen gentoypes, whereas the slow growing genotypes will result in approximately 30 to 40% higher resource utilization and environmental burden. In addition to changes in poultry production as a result of welfare regulations, future breeding goals are likely to encompass the following traits: 1) For the foreseeable future, broiler production will continue to improve as witnessed in previous years; 2) The decline in the use of subtherapeutic levels of antibiotic growth promoters in broiler feed will likely result in a drop in production, and as a result robustness is likely to attain primary importance; 3) an evolution of consumer choice drivers focusing on health, wellness, safety, and social impact, among others. In summary, in a growing and evolving marketplace, there will be room for both conventional and slower growing genotypes. While it is difficult to predict the relative representation of each type of product globally or regionally, clearly the focus from the primary breeder will be to offer genetic potential suited to all market segments while fulfilling sustainability requirements from economical, biological, welfare and environmental considerations.

Key Words: broiler breeder, broiler, consumer, welfare, genotype

Symposium: Success in the Antibiotic-Free Era Starts in the Breeders and Hatchery

569S Tipping the balance in your favor in the breeders and hatchery. J. Brake*, *North Carolina State University, Raleigh, NC.*

Chick quality has been identified as one of the most important aspects of a successful antibiotic free (ABF) program and is influenced by breeder flock and hatchery management practices that produce chicks that are relatively free of bacteria such as *E. coli*, *Salmonella*, and *Clostridia*. These bacteria must be prevented from entering the eggshell pores and from multiplication at various times thereafter up until and following hatching. This involves maintaining the cloaca in a manner that minimizes the water activity required for bacterial multiplication. Achieving this objective has been difficult due to an apparent lack of attention to the role of acid-base balance in producing dry feces and litter. Phosphate ions serve as the major renal buffer that is required to bind hydrogens produced during egg production. In the absence of sufficient phosphate buffer the kidney will route hydrogen ions through the bicarbonate buffer system to create a water molecule.The high cost of dietary phosphates has led to reduced use of phosphates and/or utilization of phytase enzymes. Excessive levels of low cost calcium has exacerbated phosphorus shortages and created wet litter. This acid-base issue can be ameliorated by appropriate dietary potassium but levels in many feedstuffs have also declined in recent years due to reduced fertilization. Poultry possess a short gastrointestinal tract (GIT) with a muscular gizzard that controls forward and reverse peristaltic action of the GIT and slows passage rate to enhance digestive efficiency that reduces nutrients available for bacteria. The fowl has to produce a dilute urine to prevent urate crystallization in the ureters. Water reabsorption takes place primarily in lower GIT under the influence of reverse peristalsis controlled by the gizzard if it has large feed particles to act upon. It has been demonstrated that the presence of large feed particles has a favorable influence on populations of both *Enterobacter* and *Clostridia*. This is due to an altered GIT pH, enhanced digestive efficiency, and less water activity. Hatching eggs will be inoculated with the bacteria present in the cloaca, which will become the first microbiota that baby chicks are exposed to.This microbiota can be favorable or unfavorable as determined by nutrition and management practices. To prevent proliferation of "bad microbiota" hatching eggs should be subjected to a relatively dry incubation environment in the presence of sufficiently high air flow to eliminate hot and cool spots within the machines while maintaining a high air flow and as low of humidity as possible during the final stages of incubation will limit the "bacterial bloom."

Key Words: antibiotic free, bacteria, chick quality, acid-base balance, incubation

570S Using egg breakouts to improve hatch and poult quality. M. Behl*, *Select Genetics, Willmar, MN.*

Egg breakout or hatch residue analysis is one of the most powerful diagnostic tools in a hatchery. Breakouts can be used to aid troubleshooting, establishing trends, or optimizing hatch results. The egg tells a story of the timeline of events from when the egg is formed inside the hen and all of the way through the hatching process. Egg breakouts do not substitute looking at the birds, but provides important insight into breeder nutrition, health, egg handling, incubating, and hatching parameters. When used in conjunction with other hatchery tools, significant progress can be made. Unfortunately, breakouts are often times overlooked and underutilized due to the lack of understanding their importance, inability to interpret them, or lack of time and resources. When hatcheries do conduct hatch residue breakout, the program is often times lackluster. The program may consist of only looking at the "poor hatches," only looking at one tray, or using generic breakout sheets. To establish trends, track embryonic changes, or identify potential areas to improve hatch, you must examine the breakouts thoroughly and frequently. To understand the "poor hatches," you must understand what embryonic mortality is in the "good hatches" and have a base understanding as to what is "normal" for specific lines and for specific weeks in lay. Breakout profiles change alongside genetic progress, therefore breakout trends and patterns are slowly changing and evolving over time. Examination of one tray is insufficient to give an accurate representation given the number of variables in a flock and or hatchery. Lastly, generic breakout sheets do not provide the specificity needed to adequately identify and resolve issues. Generic forms may actually be misleading and point you in the incorrect direction when only a portion of the story is revealed. Egg breakouts take time and resources but are an essential hatchery operation to identify normal and abnormal patterns of development. While the payback is not initially as obvious as one would like, it will pay significant dividends when problems emerge. Therefore, a concerted effort must be made to ensure breakouts are being done frequently and thoroughly. The opportunity is in the trends and details. Nowhere else can anyone find a record of events from embryo formation to hatch. Breakouts will tell you all that you need to know, you just need to listen. So many opportunities are being missed by overlooking and underutilizing egg breakouts. The answers are often times right in front of us.

Key Words: hatch residue, embryology, turkey

571S Application of vaccines in the era of antibiotic free poultry production. R. Selvaraj*, *University of Georgia, Athens, GA.*

Restriction on antibiotic growth promoters as well as therapeutic antimicrobials in poultry production is increasing the incidences of several disease outbreaks as well as increasing the incidences of food borne pathogen contamination in poultry industry. Proper application of vaccines cannot only decrease the pathogenic microbial loads but also decrease the need for using antimicrobials in poultry production. Using vaccine consistently in poultry production can lead to "herd immunity" leading to protection against unvaccinated flocks in the region. Applying vaccines against food borne pathogens in breeder population will decrease the colonization of food borne pathogens in the intestine of day old chicks. The role of vaccines in decreasing coccidial outbreaks, salmonella and *Escherichia coli* loads, necrotic enteritis severity and mycoplasma infection will be discussed. The importance of breeder vaccination, early vaccination in chicks, and uniformity of successful vaccine administration in a flock will be discussed. In addition, nutritional supplements which can improve vaccine efficacy and improve the uniformity of protection will be discussed. The role of available as well as in research and development vaccines in decreasing the antimicrobial resistance and policy decisions to apply vaccines as a tool to counter the decreased antibiotic use in poultry will be emphasized.

Key Words: antibiotic free, vaccines

572S Broiler breeder nutrition for antibiotic-free broiler production. T. Lavergne*, *Arm & Hammer Animal Nutrition, Stephenville, TX.*

As the poultry industry produces antibiotic free birds it is important to consider the role the broiler breeder plays in this type of production. The broiler breeder must produce fertile eggs with a high hatchability rate of healthy chicks. The broiler breeder needs a diet adequate in nutrients and energy, as well as one that contains good quality feed ingredients and feed additives. Without an adequate diet, hatching issues may occur such as fully developed chicks not be able to hatch, chicks dying after pipping, or chicks being weak or poor quality. The dietary considerations for broiler breeders need to begin with the pullet starter diet and continue throughout the life of the breeder. Furthermore, the management of feeding pullets and broiler breeders cannot be ignored and is key to good broiler breeder egg production. Nutritionists need to consider the availability of energy, amino acids, vitamins, and minerals in all of the feed ingredients and feed additives they use in breeder diets. There are a great deal of feed additives available for the nutritionist to use in broiler breeder diets and these include enzymes, toxin binders, probiotics, prebiotics, and antioxidants, to name a few. Thus, today the nutritionist needs to determine which feed additives are best for the broiler breeder, along with managing their feeding program and providing diets adequate in energy and nutrients. All of these factors can be influenced by the type of production system they are working with, like the antibiotic free system.

Key Words: breeder, nutrition, antibiotic-free

573S Platinum Plan: A focus on prevention for optimal health and performance. S. Ritchie*, *Canadian Poultry Consultants Ltd., Abbotsford, BC, Canada.*

The Platinum Plan is a moniker we attach to a series of "focus on prevention" programs consisting of: 1. Platinum Brooding, 2. Managing Metabolic Heat and the 3. Coccidiosis Prevention Planner. Managing poultry health in a constantly changing industry is a theme that has received increased attention and focus from international poultry organizations, academia and governments. In the not so distant past, deficiencies in brooding, were often overlooked or even tolerated. The genetic potential of the modern chicken is truly remarkable. Advances in all areas of broiler production including genetics, nutrition, management and health have resulted in continuous and significant improvements in efficiencies of production. Brooding is so critical that detailed brooding programs have been developed and continue to be developed. One of these programs, Platinum Brooding, revolves around the continuing education of producers and poultry service personal, the establishment of accurate management metrics and the establishment of a thorough, accurate and precise checklist. These management metrics are then managed and collated through dashboard feedback loops using the Platinum Brooding APP. It has been suggested that with modern genetics, today's meat yielding chickens may produce more metabolic heat; consequently, the risk associated with overheating embryos and chicks is significant. Managing Metabolic Heat is a program developed, similar to Platinum Brooding, where various metrics are collected in the hatchery, and adjustments made to ensure optimum yolk sac utilization and chick vitality. Successful coccidiosis prevention programs and strategies are multi factorial and involve strict attention to environmental management and husbandry. Coccidiosis prevention strategies involving feed additives can be very successful; however, the effectiveness of these prevention strategies can wane or fail where coccidia populations become resistant. The Coccidiosis Prevention Planner (CPP) is an instrument (software) designed to guide and record the details associated with the development and planning of an effective and efficient short, medium and long-term coccidiosis prevention program for a farm, a complex or a region. The CPP guides and records decisions made from a library of product details and records, calculates and tabulates various important metrics for long term analysis. The CPP can be customized to link and to assist other important procedures, from regulatory requirements to purchasing guidance and planning. To achieve optimum poultry health, management or husbandry must always remain front and center (centre, eh).

Key Words: broiler, brooding, hatchery, coccidiosis, husbandry

Industry Symposium: Addressing Issues Facing the Poultry Industry

574S Particle size of ingredients for broilers. A. Penz* and V. Sales, *Cargill Animal Nutrition, Porto Alegre, RS, Brazil.*

There is a gap to define how coarse the broilers prefer the particles of the diets. The general figure should be around 800 μm or more, and it is not an age of broiler related. The broilers start eating coarser particles, leaving the fines to the end. There are specific anatomic and physiological characteristics that affect fines consumption. The most important ones are selected vision capabilities, beak mechanoreceptors that react to the hardness of the particles, and small amount of saliva production. These characteristics make broilers eating more pellet than mash diets. Also, the eating process is faster and the eating energy expenditure is less, increasing the availability of energy to production, favoring the increment of body fat deposition. Still, coarse particles stimulates gizzard muscle development, important to grind the ingredients, organizing their passage to the duodenum and their digestibilities; influence duodenum-gizzard antiperistalsis, sending feed, pancreas and liver secretions back to the gizzard. The reflux improves the maintenance of the koilinic membrane of the gizzard and reduces duodenitis, buffering the acid secretion from the gizzard. This helps to maintain enterocytes integrity, important to digestibility, nutrient absorption and immune responses of the broilers. Also, antiperistalsis helps the microbial uniformity. The most common particle size determination methodology measures average geometric diameter (AGD) and geometric standard deviation (GSD). Most of the time, the particle size is measured at feed mill. It is important, for sure. However, from the feed mill to the broiler feeders, there are many events that can affect feed structure (pellet diet) and/or particles segregation (mash diet). So, systematic measurement of particle size must be taken at the farm, to register the losses that can happen from the feed mill to the farm. Regarding pellet, there is no reason to put efforts on fine gridding the ingredients once not more than 20–25% of the pellet quality is affected by particle size. Pelleting process uniformity and type of ingredients are the most important variables to be followed. When fine particles are used to improve pellet quality, that can be a good reference at the feed mill but not always represent the best at the broiler feeder. So, particle size is a feed production issue that has important consequences at the farm, affecting the performance of the broilers and the variability of the results. An holistic approach must be taken to take the advantages of this consolidated knowledge.

Key Words: particle size, ingredient, broiler

575S Antibiotic-free production in broilers: Management and nutrition. S. Pritchard*, *Premier Nutrition, Rugeley, United Kingdom.*

The UK broiler industry started to remove antibiotic growth promoters (AGPs) from diets in 1999 in response to consumer concern and the lead taken by countries such as Sweden and Denmark. In the period immediately after their withdrawal there was an increased usage of therapeutic antibiotics in response to the problems encountered in the field. This emphasizes the need for any move to antibiotic free (ABF) production to be planned and implemented gradually. Over the following 18 years the industry has and still is working to minimize antibiotic use. The British poultry meat sector published data in June 2017 showing a 71% reduction in the use of antibiotics in the last 4 years and continues as an industry to promote antibiotic stewardship. The industry mission is to ensure sustainable use of antibiotics rather than achieving a goal of zero usage at any consequence. Antimicrobial resistance (AMR) is justifiably high on the political agenda and pressure from government and the retail sector to make continued progress on this issue is immense. Since the removal of AGP producers have been offered and tried a host of products claiming to be the alternative to AGPs. The term alternative growth promoter is unhelpful. Alternatives, or in reality feed additives need to be evaluated on the grounds of quality, safety and efficacy in their own right and not thought of as AGP replacers. Producing broilers in a non-AGP environment requires an integrated approach that encompasses sound nutrition, good coccidiosis control and health management and above all good stockmanship.

Key Words: broiler, antibiotic, growth promoter

576S "All-vegetable" diets for broilers or turkeys as it relates to litter quality. S. Vieira*, *UFRGS, Porto Alegre, RS, Brazil.*

The formulation of diets for poultry without animal protein sources is done to comply with regulations (e.g., European Union), in areas where access to this sources is scarce or as an attempt to add value to the final customer. Regardless of the reason, these formulations have increased proportions of plant protein feedstuffs. In practical terms, soybean meal (SBM) is the plant source used to fulfill the protein needs only because the others are of lower availability worldwide (canola, wheat, peas...). Protein digestibility from SBM is high, however, its overall dry matter (DM) digestibility is poor mainly due to the high percentage of carbohydrates (40% on a DM basis) of very low digestibility. Around 50% of them are nonstructural in plants, including low molecular weight sugars, oligosaccharides, and small amounts of starch, while the other half are structural polysaccharides, including a large amount of pectic polysaccharides. Oligosaccharides (raffinose, stachyose and verbascose) comprise around 5% of SBM and starch with less than 1%. They are readily fermented in the small gut and frequently lead to increased anaerobiosis, frequently leading to clostridiosis. The structural carbohydrates in soy (arabinogalactan and acidic polysaccharide – pectin) in the cotyledon as well as hemicelluloses and cellulose in the hulls, are present in varying proportions of the final SBM depending on its crude protein. All vegetable diets AVD) are, generally of lower digestibility when compared with others having animal protein inclusions. Therefore, poultry fed all AVD produce greater amounts of excreta. Feet dermatitis is usually increased in flocks fed AVD, which is mainly due to stickier excreta and greater urinary flow. Changes in excreta characteristics that have a potential impact on feet dermatitis are: increased moisture due to an increased water intake driven by the high potassium contents in SBM as well as increased water retention due to the increased proportions of cellulose and hemicellulose. Pectin like carbohydrates are of low digestibility and known to lead to increased bacterial proliferation in the small intestines, which therefore, also lead to low quality excreta and increased feet dermatitis. Turkeys, requiring diets with greater potein contents, tend to have an exacerbation of poor quality litter and feet dermatitis due to the greater demand for SBM.

Key Words: all-vegetable diet, broiler, turkey, soybean meal

Symposium: Current and Future Directions for Next-Generation Sequencing of Poultry Microbiomes

577S Comparisons of microbiomes in conventional and alternative poultry production systems. M. Rothrock*, *USDA-ARS, Athens, GA.*

With the advent of new sequencing technologies and platforms, entire microbiomes are more easily characterized than ever before, while initially used more as a surveying tool to determine what microbial taxa (and their relative abundance) comprise various microbiomes, using microbiome data in a more applied manner is essential to make it more applicable to the poultry industry. While the US poultry industry is dominated by commercial, conventional management practices, upwards of 20% of the market is now comprised of poultry products produced using alternative management systems (e.g. organic, all natural, pastured). Therefore, is it not only essential that we use applied microbiomic analysis of poultry microbiomes throughout the various conventional poultry production environments, but they should also be applied to the diverse environments in these alternative management systems. For this symposium talk, I will review the applied microbiome studies that we have been performed on the different environments of the conventional poultry production system (hatchery, live production, processing plant), and then present similar data from pastured poultry flock management systems. While general trends and taxa comparisons will be discussed, applying these microbiome tools to address food safety issues will be highlighted. Also to be stressed will be the need to link this data to other important metadata (e.g. management data, physiochemistry, cultural data) to truly unlock the potential of these datasets to address the complex issues facing our increasingly diverse poultry industry.

Key Words: microbiome, conventional management, pastured poultry

578S Future directions for exploring poultry gut microbiomes: Challenges and opportunities. Y. M. Kwon* and B. Adhikari, *University of Arkansas, Fayetteville, AR.*

Gut microbiome is an integral part of a chicken in which it plays a crucial role for health, nutrition, immune responses, and disease of the host. For sustained growth of poultry industry against the challenges raised by restriction of antibiotic growth promoters (AGP), it is essential to understand the mechanisms by which gut microbiomes mediate these functions. Numerous studies have been conducted to gain understanding of the changes in gut microbiome in response to environmental factors, including diets, stresses, disease models, and rearing conditions among others. Often these studies seek to find microbiome components or features that are highly correlated with the desired phenotypic outcomes of chickens, with the goal of exploiting the knowledge to modulate gut microbiomes (e.g., probiotics) for promotion of health and growth performance or prevention of diseases. However, these comparative microbiome analyses usually provide a long list of bacterial taxa associated with certain phenotypic outcomes, and it is practically difficult to evaluate so many bacterial taxa using chicken studies for their capability to modulate chicken gut microbiomes for the desired effect in the chicken host. To advance the field and take full advantage of gut microbiome data, we will need to develop and apply innovative strategies to prioritize microbial components or features that are functionally linked to the desired phenotypic outcomes of chickens. We will discuss the future directions of poultry microbiome studies to realize these goals, which include advanced experimental design, improved algorithms to process MiSeq data for 16S rRNA gene sequences, functional insights via shotgun metagenomics, and more accurate quantitative microbiome profiling.

Key Words: poultry, gut microbiome, 16S rRNA gene, shotgun metagenomics, probiotics

579S Microbiome and pathogen interaction with the immune system. M. Kogut*, *USDA-ARS, College Station, TX.*

The intestinal tract harbors a diverse community of microbes that have co-evolved with the host immune system. Although many of these microbes execute functions that are critical for host physiology, the host immune system must control the microbial community so that the dynamics of this interdependent relationship is maintained. To facilitate host homeostasis, the immune system ensures that the microbial load is tolerated, but anatomically contained, while remaining reactive to microbial invasion. Although the microbiota is required for intestinal immune development, immune responses regulate the structure and composition of the intestinal microbiota by evolving unique immune adaptations that manage this high bacterial load. The immune mechanisms work together to ensure that commensal bacteria rarely breach the intestinal barrier and that any that do invade should be killed rapidly to prevent penetration to systemic sites. The communication between microbiota and immune system is mediated by interaction of bacterial components with pattern recognition receptors expressed by intestinal epithelium and various antigen-presenting cells resulting in activation of both innate and adaptive immune responses. Interaction between microbial community and host plays a crucial role in the mucosal homeostasis and health status of the host. In addition to providing a home to numerous microbial inhabitants, the intestinal tract is an active immunological organ, with more resident immune cells than anywhere else in the body, organized in lymphoid structures called Peyer's patches and isolated lymphoid follicles such as the cecal tonsils. Macrophages, dendritic cells, various subsets of T cells, B cells and the secretory IgA they produce all contribute to the generation of a proper immune response to invading pathogens, while keeping the resident microbial community in check without generating an overt inflammatory response to it. IgA-producing plasma cells, intraepithelial lymphocytes and γδT cell receptor (TCR)-expressing T cells are lymphocytes that are uniquely present in the mucosa. In addition, of the γδT cells in the intestinal lamina propria, there are significant numbers of IL-17-producing T (Th17) cells and regulatory T (Treg) cells. The accumulation and function of these mucosal leukocytes are regulated by the presence of intestinal microbiota which regulate these immune cells and enhance the mucosal barrier function allowing the host to mount robust immune responses against invading pathogens, and simultaneously maintains immune homeostasis.

Key Words: microbiome, immunity, tolerance, inflammation

580S Effects of feed additives on poultry cecal microbiome. S. I. Lee[1], S. Ricke[2], and S. H. Park*[1], *[1]Oregon State University, Corvallis, OR, [2]University of Arkansas, Fayetteville, AR.*

Poultry products are some of the most popular food products throughout the world. Gastrointestinal tracts of poultry possess pathogenic bacteria

such as *Salmonella* and *Campylobacter* without disease symptoms thus their presence has been frequently implicated in outbreaks in humans when consumed contaminated poultry products. As consumers become more interested in food safety and the consumption of poultry products increase, contamination of those bacteria is a major concern in poultry related industries. To not only reduce the pathogenic bacteria but increase beneficial bacteria in the gastrointestinal tracts of poultry, several feed additives such as prebiotics and antimicrobials have been widely utilized. To date, the microbiological analysis in the gastrointestinal tracts of poultry associated with food safety has been focused on the efficacy of feed additives in reducing *Campylobacter* or *Salmonella*. However, there have only been limited studies focused on microbiome and microbial communities before and after antimicrobial treatments. The next generation sequencing (NGS) technology has been developed over past decade and decreasing cost per sequence has allowed for enhanced characterization and profiling of microbiota in complex ecosystems. High-throughput NGS platforms based on 16S rRNA gene amplicons, called microbiome sequencing, can serve as a useful technique to delineate the more complex aspects of the microbiome in cecal contents of poultry. Microbiome sequencing is the most recent technique to identify all microorganisms including unculturable microorganisms in a certain complex ecosystem such as gut and soil. Identification of overall microbial populations in ecosystems such as a chicken cecal contents have allowed us deep insights for bacterial functions and interaction between hosts and bacteria. High-throughput NGS platforms based on 16S rRNA gene amplicons can serve as a phylogenetic markers to define the more complex aspects of the microbiome in poultry and poultry products. Documenting how microbial community changes from a phylum to a genus level may help achieve in-depth understanding of the microbial dynamics in poultry with antimicrobial treatments.

Key Words: poultry, microbiome, next-generation sequencing

581S Application of microbiome analyses in poultry processing. S. Ricke*, *University of Arkansas, Fayetteville, AR.*

Poultry processing involves multiple steps to generate poultry meat for retail distribution. During processing, poultry carcasses are exposed to various environmental conditions including variations in moisture levels and application of antimicrobials. Consequently, bacterial loads change as environmental conditions change. Monitoring carcass bacterial loads has traditionally been done by conventional microbial plating and enumerating the colonies on these plates. Such measurements allow for baseline data to assess antimicrobial efficacy and overall processing performance. Further delineation via selective plating of specific non-pathogenic microorganisms that parallel behavior and physiology of foodborne pathogens can be used as indicator organism data to predict how the corresponding pathogen would respond is also considered useful information for assessing processing performance. However, more detailed changes in carcass microbial populations has not been possible with these microbial monitoring approaches. Next generation sequencing of the microbiome has led to in-depth microbial taxa profiling and diversity comparisons among different microbial communities from different sources. Microbiome sequencing has been applied to poultry processing carcass rinsates and compared with conventional plating methods. These studies have revealed major shifts in bacterial taxa as carcasses proceed through the steps of processing with different groups becoming predominant depending on the specific processing step. As more becomes known about these microbial populations based on microbiome sequencing, opportunities to identify more representative indicator organisms and more precisely track microbial shifts in populations related to a specific processing step are becoming possible. This presentation will provide an overview of approaches and insights that processing microbiome data has revealed thus far.

Key Words: poultry processing, indicator organism, next-generation sequencing, microbiome, foodborne pathogen

Symposium: Amino Acids and Low-Protein Diets: Benefits for Performance, Meat Quality, Environment, Health, and Welfare of Poultry Birds

582S Update on amino acid requirements for broiler chickens. P. Tillman*[1] and W. Dozier[2], [1]*Poultry Technical Nutrition Services LLC, Buford, GA,* [2]*Auburn University, Auburn, AL.*

Broiler production and nutritional practices are rapidly changing due to demands for Raised Without Antibiotics meat, improved meat quality, changes in either conventional or to heritage breeds, and concerns over environmental impact, animal health and animal welfare. All of these changes can alter the optimal amino acid "requirement," depending upon whether the goal is improving average daily gain, feed conversion, yield, profitability or one or more of these goals. Raised Without Antibiotics production, often accompanied with a transition to all vegetable-based diets, places more emphasis on "gut health" and perhaps a change in ingredients to be considered. The transition to vegetable-based diets may also alter the order of amino acid limitation and requires more emphasis be placed upon key amino acids such as valine and glycine. White-striping, wooden breast and other breast myopathies may impact targeted growth rates, market bird weights and thus days to market until resolved through genetic selection or some other means. Increased growth rate and feed efficiency of modern "fast-growing" genotypes and a trend toward the opposite end of the spectrum (i.e., "slow-growth") both emphasize the need to better understand the amino acid and energy "requirements" of these vastly different broilers. As there is no single "requirement" for any given amino acid, as this depends up on the desired outcome – the concept of meeting more than one of these targets is daunting. Each of these changes, can have positive and / or negative impacts upon each of the aforementioned goals and ultimately on profitability. In addition, the role of several if not every essential amino acid can be involved in most of these key areas of interest. This paper will provide a North American summary of some of these current research areas, with a focus on the broilers "requirements" and some functional properties of certain amino acids.

Key Words: amino acids, reduced protein, requirements, broiler

583S Functional amino acids in poultry nutrition: Recent developments and practical applications. G. Wu*, *Texas A&M University, College Station, TX.*

Based on growth or nitrogen-balance studies, amino acids (AA) had been classified traditionally as nutritionally essential (EAA) or nonessential (NEAA) for poultry. A limitation of the previous classification of AAs as EAA or NEAA is that regulatory roles for AAs in animal metabolism, immunity and health had not been considered in dietary formulation. For example, glutamate is quantitatively the most important energy substrate for enterocytes of 0 to 42-d-old chickens, whereas glutamine is a major metabolic fuel in chicken skeletal muscle. Growing evidence shows that some EAA (e.g., arginine, glycine, leucine and tryptophan) and NEAA (e.g., glutamine) can activate the mammalian target of rapamycin (mTOR) cell signaling to promote protein synthesis and inhibit proteolysis in tissues. Certain AAs (e.g., arginine, cysteine, glutamine, glutamate, glycine, proline, and tryptophan) also play important roles in regulating gene expression, male and female fertility, acid-base balance, redox signaling, detoxification of xenobiotics and endogenous metabolites, neurotransmission, and immune responses. Furthermore, some AAs (e.g., threonine and tryptophan) are crucial for intestinal mucosal integrity and anti-oxidative signaling. These findings lead to a new concept of functional AAs (FAAs), which are defined as those AA that regulate key metabolic pathways to improve the health, survival, growth, development, and reproduction of animals. As examples for translating this novel concept to feeding practices, supplementing FAAs to conventional corn- and soybean meal-based diets enhances weight gain, skeletal muscle mass, and nutrient absorption and utilization, while reducing abdominal fat, plasma lipids, and mortality in broilers, particularly under conditions of inflammation, infection, and heat stress. Improvements in poultry feed efficiency and productivity through the use of FAAs will not only reduce the contamination of soils, groundwater, and air by excessive manure, but will also help sustain poultry agriculture to produce high-quality protein for the expanding population in the face of diminishing resources worldwide.

Key Words: amino acid, poultry nutrition, growth, immunity, health

584S Health and welfare improvements with low-protein diets and amino acids—Importance of threonine. L. Star*[1] and W. Lambert[2], [1]*Schothorst Feed Research, Lelystad, the Netherlands,* [2]*Ajinomoto Eurolysine S.A.S., Paris, France.*

Reduction of crude protein (CP) in poultry diets is of interest for several reasons. First, there is an economic interest. Lower CP level in the diet will reduce the dependency on foreign feedstuffs, reduce use of GMO products, and reduce feed prices. Second, lower CP level is of interest for welfare. Excess of CP leads to a greater need of water to achieve efficient nitrogen excretion and this might results in degradation of litter quality and increase of foot pad lesions. Besides, a high CP level is a predisposing factor for the occurrence of necrotic enteritis. Third, reducing CP level in the diet is of interest for the environment. CP levels in the diet will have a direct effect on excretion of N. Excreted N can be very quickly converted to ammonia (NH_3). Reduction of CP in poultry diets is possible, but we should be aware that AA will not become limiting. If one essential AA does not meet the bird's requirement, it will be limiting for production performance. Therefore, the ideal AA concept should be applied to make sure that essential AA are provided by feed. Several feed grade AA are routinely incorporated into poultry feeds. These AA are available at a price that permits their use in least-cost formulated diets. With these feed grade AA it is already possible to reduce CP in poultry diets and thereby reduce N excretion. It is expected that in the near future, the next limiting AA will become available at reasonable price for poultry feed. This will result in an even more accurate supply of AA to meet the need requirements of the bird. Results on performance of birds fed low CP diets are not consist in literature. Used levels of CP, essential AA and non-essential AA differ between studies. One assumption is that digestible glycine (dGly) might be limiting in low CP diets, and thereby digestible threonine (dThr), because Gly can be metabolized from Thr. Therefore, the sparing effect of dThr on dGly in practical broiler diets was investigated. Besides, Thr is particularly important for mucin synthesis and maintenance of gut barrier integrity. Standard dThr-to-digestible lysine ratio suited for healthy birds can cause Thr deficiency in birds with intestinal problems resulting in loss of performance after infection. Three broiler trials to test dThr level during necrotic enteritis infection. The studies showed the importance of dThr in low CP diets as well as in broilers with intestinal health problems. It was concluded that at a sufficient dThr level dGly was not limiting, and to support broilers with intestinal health problems, dThr can be supplied at a higher level.

Key Words: low crude protein, threonine, glycine, intestinal health, excretion

585S Use of feed-grade amino acids in low protein diets: Towards a more sustainable broiler production? B. Meda*[1], P. Belloir[2], W. Lambert[3], M. Lessire[1], and S. Tesseraud[1], [1]*INRA, Nouzilly, France,* [2]*Ecole d'ingénieur de Purpan, Toulouse, France,* [3]*Ajinomoto Eurolysine, Paris, France.*

In Europe, consumers and citizens are questioning the sustainability of broiler production, as they are waiting for affordable poultry meat produced with low environmental impacts and high animal welfare. The origin of the feed ingredients is also a growing concern since a more local production, without GMOs, is expected. In this context, reducing dietary crude protein could be an interesting strategy to simultaneously decrease nitrogen excretion of birds and the European dependency to GMO soybean meal importations. The aim of this study was thus to investigate the effect of decreasing dietary crude protein (CP) in finishing broiler on animal performance, meat quality and environmental impacts. PM3 Ross male broilers were reared together between 1 and 20d of age. At d21, they were randomly distributed in 24 floor pens (8 pens per treatment; 38 birds per pen) and fed until d35 (slaughter) with diets formulated with an amino acid (AA) profile based on the ideal protein concept. More specifically, the minimum AA:Lys ratios proposed by Mack et al. (1999) were used with modifications for Thr and Arg (Thr:Lys ratio increased from 63 to 68% and Arg:Lys ratio decreased from 112 to 108%, respectively). CP contents of experimental diets were 19, 17.5 and 16%, respectively. With CP reduction, the proportion of soybean meal in diets decreased (28, 24 and 18%, respectively), while those of corn and feed-grade AA increased (for AA: 0.23, 0.48 and 1.19%, respectively). CP reduction did not affect body weight (BW) gain, feed efficiency or breast meat yield but abdominal fat increased. Meat quality criteria responded to dietary CP reduction with higher ultimate pH and lower lightness and drip loss, but these variations were considered acceptable for meat preservation or processing. Nitrogen excretion decreased with CP (−12% per CP point) and so did volatilization. A life cycle analysis was also carried out to compare the environmental impacts of one kg of BW at farm gate, with 19 or 16% CP in finishing diet respectively. CP reduction decreased climate change, eutrophication and energy use impacts by 8, 7 and 1%, respectively. For acidification, the impact per ton of finishing diet increased by 4%, but the final impact decreased by 5%, due to decrease in nitrogen excretion and volatilization. In conclusion, CP reduction in finishing broilers is therefore possible using an adapted AA profile and feed-grade AA. Such a feeding strategy could improve the sustainability of broiler production with similar animal performance, no detrimental effect on meat quality, lower environmental impacts and a reduced use of GMO soybean meal.

Key Words: protein, amino acid, sustainability, environment, soybean meal

586S Modulation of chicken meat quality by protein and AA nutrition. C. Berri*[1], P. Belloir[2], M. Lessire[1], S. Metayer-Coustard[1], and S. Tesseraud[1], [1]*BOA, INRA, Université de Tours, Nouzilly, France,* [2]*Ecole d'ingénieur de Purpan, Toulouse, France.*

Poultry products are mainly consumed as cut and processed products. Therefore, it is no longer enough for broilers to have high slaughter yields but sensorial and functional characteristics of meat must be taken into consideration to satisfy the demands of both processor and consumer. This review focuses on the recent advances showing that nutrition can be an effective tool to control muscle development and meat quality in poultry. In particular, the intake of protein and amino acids, which largely determines muscle growth and yield, may affect several molecular pathways with significant consequences on muscle postmortem metabolism and meat quality. Indeed, the amino acid supply during the finishing period or just before slaughter can shape the energy reserves of the muscle with a significant impact on meat quality, including color, processing yield and susceptibility to oxidation. Beyond the control of muscle metabolism, protein intake will also be crucial in controlling the molecular pathways that influence muscle fiber growth and integrity. Thus, recent studies show that in modern heavy strains, improving meat yields by nutrition can also lead to poor meat quality, and in the most severe cases to the onset of myodegenerative defects, such as white striping or wooden breast. Therefore, it is essential to rethink poultry nutrition and optimize dietary supplies (quantitative and qualitative) over time and in function of production targets.

Key Words: meat quality, amino acid, muscle, postmortem metabolism

587S Effect of low-protein diets on nitrogen utilization, daily water consumption, and litter quality in broilers through meta-analysis approach. M.-P. Montminy*[1], W. Lambert[2], and O. Cirot[2], [1]*Université Laval, Quebec, QC, Canada,* [2]*Ajinomoto Eurolysine, Paris, France.*

Reducing dietary crude protein (CP) is the most efficient strategy to reduce nitrogen (N) excretion by broilers that has also been reported to improve litter quality through reduction in water consumption and litter moisture (LM). Given the high amount of data available in literature on dietary CP reduction in broilers, a meta-analysis was performed to quantify the impact of reducing CP on N balance (N intake, excretion, retention, and retention efficiency), daily water consumption (DWC), LM and uric acid in serum or plasma express in percentage of the highest CP value (UA). The effect of the trial has been tested as a random effect and those of supplying the indispensable amino acids (IAA) at their requirement or not (IAAreq and IAAnot-req) and bird age (0–21 or 21–42 d) were also investigated as fixed effects in this meta-analysis. Based on a data set of 116 trials for N balance criteria, the analysis revealed that reducing the dietary CP linearly decreases N intake ($P < 0.001$; $R^2 = 99\%$) and N excretion ($P < 0.001$; $R^2 = 99\%$) with a higher effect for 21–42 d than 0–21 d broilers for excretion (CP × Age, $P < 0.001$). Besides, reducing dietary CP increases linearly the efficiency of N utilization ($P < 0.001$; $R^2 = 98\%$) without impact of IAA level or age, meaning that age effect observed on N excretion is due to a higher consumption. Nitrogen efficiency of broilers can thus be increased by 2.3% per % of dietary CP reduction. Based on a data set of 22 trials for DWC, the analysis revealed that DWC of broilers (expressed in proportion to the highest CP level within trial) decreases linearly with the reduction of dietary CP level ($P < 0.001$; $R^2 = 72\%$); increasing CP by 1 point % increases DWC by 2%. Regarding LM, based on 12 trials, the response to CP ($P < 0.001$; $R^2 = 67\%$) showed that the LM of broiler might be reduced by 2.4% per point % of dietary CP reduction. Finally, based on 28 trials, UA increased in a linear ($P = 0.02$) and quadratic ($P = 0.04$) manner ($R^2 = 57\%$) with increasing dietary CP, probably due to an excess of amino acid, until a plateau reach around 20 to 21% CP. Given the low R^2 obtained for DWC, LW, and UA, other X variables maybe implicated in these responses. This meta-analysis helps to quantify the effects of reducing dietary CP levels on N balance and on the litter quality of broilers. The combined reduction of N excretion and LM when reducing the dietary CP level may decrease the occurrence of footpad dermatitis by improving the litter quality. These results are useful to formulate low CP diets for a more sustainable broiler production.

Key Words: low protein, amino acid, broiler, meta-analysis

Symposium: Novel Applications of Light Applied to Poultry Production: Influencing Growth, Behavior, and Biosecurity

588S Impact of day length on health and welfare of broilers and turkeys. K. Schwean-Lardner* and T. Shynkaruk, *University of Saskatchewan, Saskatoon, SK, Canada.*

The inclusion of a dark period in a broiler or turkey lighting program has become common over the recent years, however there is still disagreement for its necessity. Recently, the requirement of a minimum of 4 consecutive hours of darkness was mandated in the 2016 *Canadian Codes of Practice for the Care and Handling of Hatching Eggs, Breeders, Chickens, and Turkeys*, but not without controversy. The evidence for improvement of welfare when a minimum of 4 h of darkness is included is substantial. This includes improved health leading to reduced morbidity and mortality, improved mobility, less detrimental impacts on ocular health, wider behavioral repertoire, and reductions in footpad lesions. Several mechanisms have been identified that may lead to these welfare improvements. These include altering the time of growth, level of exercise, behavioral patterns of feeding, and the physiological response to darkness, including development of diurnal rhythms in hormones and core body temperature.

Key Words: light, broiler, turkey, welfare

589S Lighting program effects on sexual maturity and early egg size in laying hens. I. Rubinoff*, *Hy-Line International, Dallas Center, IA.*

The objective of this analysis is to better understand the impact of the genotype by environment effect of lighting programs on the age of sexual maturity and early egg size in laying hens. The approach of the Hy-Line Research Department is to maintain sexual maturity between 19 and 21 weeks for all commercial lines. Despite the consistency of sexual maturity on the pedigree level, there is a variance of reaching 50% production around the world due to lighting, nutrition, and management differences. Three sources of data were used to help understand the issue, the Hy-Line commercial database, test birds on the Hy-Line research farm, and Hy-Line lighting trials. The commercial database was utilized to observe the variance of sexual maturity and early egg size in birds around the world. Trial 1 studied the effect of rapid, moderate, and slow stepdown programs on W-36 and HLB sexual maturity and early egg size. Trial 2 examined the effect of 24/12, 12/12, and moderate stepdown programs on W-36 and W-80 sexual maturity and early egg size. Trial 3 examined the effect of 15 week versus 17 week light stimulation on HLB and HLB+ pullets with the same stepdown program. Commercial data – the average age of 50% hen day production falls between 20 and 21 weeks of age with a variance of ± 2 weeks. The greatest variance in HD% is at 20 weeks for both Brown (SD ± 25.7%) and W-36 (SD ± 19.8%) flocks. The greatest variance in egg size is at the beginning of lay. For Brown the average is within one standard deviation of the mean for large eggs (56.7 g) by 22 weeks of age (54.6 g ± 2.42 g) and for W-36 by 25 weeks of age (55.2 g ± 2.11 g). Trial 1 showed that rapid stepdown programs have a significantly earlier age of sexual maturity, slow stepdown programs have significantly larger egg weights at the beginning of lay. Trial 2 showed that 12/12 programs have significantly heavier body weights and significantly lower egg weights than normal program. 24/12 programs have significantly lighter egg weights and statistically similar egg weights with the normal program. The statistics are being run on Trial 3. Numerically there is an impact of genetic lines on egg size and early differences in production and body weights related to the age of stimulation. Lighting programs and management play a significant role in maximizing the economic and genetic potential of commercial laying hens. The same genetics around the world can have very different results based on the local conditions and management approaches.

Key Words: light, physiology, stimulation, sexual maturity, egg size

590S Using light to mitigate odor and improving air quality. J. Koziel*, D. Maurer, W. Zhu, and W. Jenks, *Iowa State University, Ames, IA.*

Technologies for mitigating odor, gaseous emissions, and for improving indoor air quality at animal production systems are of interest to producers, the public, and regulatory agencies. In our previous lab-scale studies (Rockafellow et al., 2012) we demonstrated that ammonia (NH_3) abatement is viable at the proof-of-principle level using photolysis with 185/254 nm low pressure mercury UV lamps. However, exposure to bactericidal short wave UVC can also be toxic skin and eyes. Black UV (UVA, long wave) light is by far less toxic to people. Zhu et al. (2017) showed that the use of a photocatalytic coating on surfaces subjected to black UV reduced emissions of key odorant compounds relevant to the animal production industry. Most recently, an on-farm pilot-scale experiment was conducted at a commercial swine barn to evaluate a photocatalytic coating on surfaces subjected to black UV under field conditions. A flow-through reactor was constructed with a TiO_2-based photocatalytic coating on the interior surfaces and black UV fixtures. The reactor was deployed to treat a slip stream from a swine barn fan. Gas samples were collected at the inlet (control), the midpoint (half treatment) and the outlet (treatment) of the reactor. Significant reductions in emissions were observed for 4-methyl phenol (a.k.a. *p*-cresol; 22%), odor (16%) and nitrous oxide (N_2O, 9%). A significant increase in CO_2 (3%) was also measured. Results show that the TiO_2-based photocatalytic coating and black UV light are effective is mitigating odor, *p*-cresol (responsible for the characteristic downwind nuisance odor experienced far downwind), and an impactful greenhouse gas (N_2O).

Key Words: odor, animal production systems, UV light, air quality, photocatalysis

591S Use of germicidal UV light to improve biosecurity. A. Stephan*[1] and J. VandenBoom[2], *[1]ONCE Inc., Plymouth, MN, [2]University of Minnesota, St. Paul, MN.*

The objective of this study was to develop and evaluate the effectiveness of light-based disinfection technologies for use on agricultural surfaces and eggs. Light represents a subset of electromagnetic radiation that is perceptible by living organisms. The wavelength and energy of this radiation is especially well-suited for interaction with biological molecules. Short-wavelength, high-energy UV C radiation is readily absorbed by covalent bonds, which results in protein and DNA damage. UVC has thus been used for over a century as a germicidal treatment of medical equipment. Nevertheless, adoption of UVC by livestock and poultry producers has been limited due to technical limitations of the technology. More recently, longer-wavelength UV A as well as human-visible light has been shown to generate reactive oxygen species when absorbed by photo-activatable pigment molecules. This technology is

known as photodynamic therapy or photoactivation. For this study, a laboratory strain of *E. coli* was used as model for gram-negative bacteria- the predominant clade of bacteria contaminating table and hatchery eggs causing spoilage and foodborne illness. The effectiveness of killing by various light sources as photoactivatable compounds was evaluated. *E. coli* strain MM294 (genotype: F–endA1 hsdR17 (rK– mK+) glnV44 thi-1 relA1? rfbD1? spoT1?), was grown in standard Luria-Bertani medium to stationary phase. Petri dishes and eggshells were inoculated with a known number of viable bacteria. Standard assays varied the light treatment (light vs dark) and the photo-activater (+/ photoactivatable compound). Experiments varied the wavelength, intensity, exposure time, concentration of photoactivatable compound, and type of photoactivatable compound. The bacterial inoculum was recovered and plated in 10-fold serial dilutions for quantification. Bacterial reductions were expressed in log10 units and were evaluated for significance by 2-way ANOVA for light and chemical treatments. Bacteria were readily reduced when both light and photoactivatable compounds were present. Optimal wavelengths depended on the type of photoactivatable compound used and ranged from 365 nm for TiO2 to 405 nm for protoporphyrin IX. Bacterial reductions of > 4 logs were readily achievable under sufficient irradiance and exposure times. Use of UV and near-UV light represents a promising novel method for disinfection for table- and hatchery eggs as well as other agricultural surfaces.

Key Words: photobiology, egg sanitation, antibiotic-free, hatcheries, photoactivation

592S Behavior and production responses of W-36 chicks to supplementary UVA light. K. Liu*[1,2], K. Wang[1], T. Fei[2], L. Chai[1], and H. Xin[1], *[1]Iowa State University, Ames, IA, [2]University of Pennsylvania, Kennett Square, PA.*

UVA (315–400 nm) light perception is an essential part of poultry vision, which may be used to modify behavioral traits of birds such as feeding, peer recognition, and social encounters. It may be of socio-economic significance to provide certain amount of UVA light in poultry production, particularly in modern facilities where artificial lighting is the only light source for the birds. The objectives of this study were to assess behavior and production responses of W-36 chicks reared under LED light with or without different levels of UVA supplementation (0%, 5%, 10%, and 15%), i.e., LED vs. LED+UVA. For the behavior response assessment, a total of 108 d-old chicks in 18 groups over 9 batches were assessed for their choice via preference test. For each group, the birds were individually identified with different colored marks on the head. Each group of chicks involved an 8-d preference test, during which the birds could move freely between 2 inter-connected compartments that contained LED and LED+UVA, respectively. Trajectory of each bird was tracked using computer vision. Time spent and feed intake by the birds under each light condition were measured. For the production response assessment, a total of 270 d-old chicks in 18 groups over 9 batches were used to assess the effects of the UVA supplementation on feed use, body weight gain, and eye condition of the chicks. For each batch, 2 groups were randomly assigned to 2 compartments, one with LED and the other with LED+UVA. Feed use in each compartment was manually weighed daily. Body weight of individual chicks was weighted at the placement and the end of the test. Eye condition was assessed by a board-certified veterinary ophthalmologist at the end. The following results were found. In the scenario of 0% *vs*. 5% UVA, the chicks spent significantly lower amount of time under LED+UVA than under LED (45.6% *vs*. 54.4%), but had comparable feed use under both light conditions. In the scenario of 0% *vs*. 10% UVA, the chicks showed similar amount of time spent and feed use. In the scenario of 0% *vs*. 15% UVA, the chicks spent significantly higher proportion of time (61.3% *vs*. 38.7%) and consumed significantly more feed (60.5% *vs*. 39.5%) under LED+UVA than under LED. Chicks had comparable growing performance and no eye pathology was detected at any UVA supplementation levels. The study demonstrates the attracting effect of UVA light at 15% inclusion rate under LED illumination on chicks in terms of time spent and feed use. A large-scale and long-term study to further verify the positive effects of UVA inclusion seems warranted.

Key Words: poultry lighting, UVA radiation, computer vision, light preference, growing performance

593S The effect of targeted monochromatic photostimulation on growth and reproduction of broiler birds. I. Rozenboim*[1], M. El Halawani[3], Y. Chaiseha[4], N. A. Cohen[1], S. Zaguri[1], Y. Bartman[1], L. Dishon[1], and T. Porter[2], *[1]Hebrew University of Jerusalem, Rehovot, Israel, [2]University of Maryland, College Park, MD [3]University of Minnesota, St Paul, MD [4]School of Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand.*

Targeted illumination in modern poultry management is becoming a common practice to increase productivity. Broilers photostimulated with green light (GL) in-ovo or post hatch were heavier than dark incubated birds. In the present study, we defined the cellular and molecular events associated with the effect of in ovo and post hatch GL photostimulation on muscle growth. We found that GL photostimulation has a stimulatory effect on proliferation and differentiation of satellite cells and a promoting effect on the uniformity of the muscle fibers in the early post-hatch period. We gathered evidence to support these findings. In ovo GL photostimulation increased plasma growth hormone (GH) and prolactin (PRL) levels, as well as mRNA levels for hypothalamic growth hormone releasing hormone (GHRH), both liver growth hormone receptors (GHR), and liver insulin-like growth factor-1 (IGF-1). Furthermore, new evidence suggests that the critical embryonic period for targeted GL photostimulation is between 15 and 18 d of incubation. Both retinal and extra-retinal photoreceptors were detected at d 14 of incubation, and mRNA levels were altered by different photostimulation wavelengths. Many avian species are photoperiodic and respond to long photoperiods with an activation of the reproductive axis. Retinal and extra-retinal photoreceptors were suggested to be involved in the detection of daily or seasonal changes. In birds subjected to a gonad stimulating photoperiod, long wave radiation (630-780 nm) penetrates the tissue and directly acts on hypothalamic extraretinal photoreceptors to stimulate reproductive function. In contrast to the stimulatory effect of long wave radiation on reproductive activity, activation of retinal photoreceptors by visible radiation in the green-yellow bands of the spectrum (545-575 nm), appears to be inhibitory to reproduction. In a recent studies conducted on broiler breeders flocks we found that extraretinal photostimulation combined with nonphotostimulatory conditions to the retina caused a significant elevation in reproductive activity manifested by increased hypothalamic GnRH-I and pituitary LH and FSH mRNA expression, increased plasma LH and gonadal steroids, increased egg production, improved semen quality parameters, and more chicks/breeder hen. We suggest that targeted illumination plays a pivot environmental stimulatory role in both meat type birds and breeders.

Key Words: target photostimulation, reproduction, growth

Symposium: From the Indigestible Fraction to the Global Feed Enzyme Approach

594S Non-starch polysaccharides and fibers: From direct to indirect effect on global digestibility. K. E. Bach Knudsen*, *Aarhus University, Tjele, Denmark.*

Dietary fiber represents the fraction of carbohydrates – non-starch polysaccharides, resistant starch and non-digestible oligosaccharides – along with the non-carbohydrate polyphenolic ether lignin that is not digested by endogenous enzymes in the small intestine of non-ruminant species. Because of the non-digestibility, the dietary fiber fraction will have a direct negative effect on the digestibility and traditionally fiber, irrespective the method used for analyses – crude fiber, neutral detergent fiber, acid detergent fiber, total dietary fiber – has been considered a marker for low digestibility and nutrient utilization of feed for animals. The dietary fiber fraction, however, also possesses several indirect effects with consequences for nutrient digestibility and utilization: 1) influence on swelling, water binding capacity and viscosity of digesta with consequences on rate and extent of digestion and absorption of nutrients in the small intestine and endogenous secretion, 2) encapsulation of potentially available nutrients such as high quality proteins and minerals in the aleurone cells and starch in some legumes and 3) influence on passage rate in small and large intestine. All in all, the dietary fibers has direct and indirect effects on digestion, absorption and metabolism at all sites of the gastrointestinal tract with consequences for the global nutrient digestibility and utilization.

Key Words: non-starch polysaccharides, fiber, digestibility, poultry

595S Formulating on indigestible fraction: A chance for improving sustainable animal protein production. O. Adeola*, *Purdue University, West Lafayette, IN.*

Appropriate use of exogenous enzymes in feeds requires strategic reductions in dietary nutrient and energy levels, as well as careful choice of feed ingredients to capture economic benefits of the various enzymes. Interest in the use of exogenous enzymes in poultry feeds is ever increasing and unique attention should be focused on substrates to match added enzyme with its substrates that form enzyme-substrate complex for subsequent hydrolysis. As a consequence of the hydrolysis reaction, nutrients released can be absorbed and used for metabolic needs. This emphasizes the importance of adequate information on the indigestible fraction in feeds to optimize the opportunity for gaining economic value of enzymes. Linear feed formulation software have limited data on the proportions of fractions of ingredients that are indigestible in nutritional. The dearth of information on indigestible fractions in feeds limits the benefits of exogenous enzymes supplementation. Providing insights into the indigestible components such as phytate and fiber in the poultry feed and their interaction is key for capturing the value of exogenous enzymes that hydrolyze and release nutrients for use by poultry. It is incontrovertible that phytase is consistently efficacious in releasing orthophosphate from phytate contained in plant-based feeds for poultry. Because enzymes such as phytase hydrolyzes approximately 50% of dietary phytate, there is considerable room for the development of strategies that will further improve hydrolysis of phytate. Carbohydrases on the other hand are equivocal based on several factors including a lack of information on amount of structure of non-starch polysaccharides in feed. Regardless, the use of exogenous enzymes in diets of non-ruminants continues to be promising for a variety of reasons that hinge on sustainability, economics, and environmental.

Key Words: carbohydrase, indigestible fraction, non-ruminant, phytase, protease

596S Moving forward in amino acid nutrition: Key needs, digestibility, and exogenous enzymes. M. Kidd*[2], P. Cozannet[1], and A. Bello[1], *[1]Adisseo France S.A.S, Malicorne, France, [2]Center of Excellence for Poultry Science, Fayetteville, AR.*

Throughout the latter half of the twentieth century methionine and lysine sources successfully entered broiler least cost formulation with numerous constraints: namely, feed grade amino acid ingredient maximums, CP minimums, ingredient variability, and poor ingredient amino acid digestibility data.During the last few years of the twentieth century, L-threonine entered broiler formulas in the United States.Two key nutritional factors contributed to the use of L-threonine in broiler diets: reliable ingredient digestibility data and an understanding of the digestible threonine to lysine need in broilers of various ages.Formulation addition of other feed grade amino acids going forward (i.e., L-Arg, L-Val, L-Ile, Gly, or L-Gln) will require precise knowledge of enteric amino acid needs and amino acid digestibility data as impacted by environment and exogenous enzymes.As feed grade amino acids are considered 100% digestible, the amino acid supply from the indigestible fraction of cereals and oilseeds, especially in the presence of exogenous enzymes, must be quantified.For example, phytase increases amino acid digestibility by acting on protein-phytate molecules bound in both binary and ternary forms whereas non starch polysaccharide enzymes act on fiber-protein complexes thereby increasing amino acid digestibility. Going forward, measuring amino acid digestibility in low CP diets of industry relevance with and without exogenous enzyme combinations will be key to assess amino acid digestibility, overall N balance, and simultaneous enzyme benefit on indigestible protein substrates.

Key Words: amino acid, enzyme, nitrogen, digestibility

597S Enzymes: From their pro-prebiotic role to gut health benefits. N. Yacoubi*[1], L. Saulnier[3], E. Bonnin[3], E. Devillard[1], R. Ducatelle[2], and F. Van Immerseel[2], *[1]Adisseo, Malicorne, France, [2]Faculty of Veterinary Medicine, Merelbeke, Belgium, [3]INRA, Nantes, France.*

Feed enzymes (Enz) may represent an effective method to replace antibiotic as growth promoters in animal production. Their mechanisms of action are complex and consist on direct and indirect effects. Enz allow breaking down cereal cell walls NSP, including arabinoxylans (AX), help releasing encaged-nutrients but also decreasing NSP average molecular weight and intrinsic viscosity and by consequence improving feed digestibility and optimizing the feed passage rate in the GIT. The short-chain polysaccharides and oligosaccharides produced by enzymatic degradation can also show prebiotic properties. A prebiotic is a selectively fermented non-digestible dietary ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health. The advantage of NSP-degrading enzymes is that they allow transforming the cereal-NSP, considered as anti-nutritional factors, to prebiotic-like components and take benefit of the associated effects of steering the intestinal microbiota toward one with a dominance of beneficial bacteria. Butyrate production is for instance known to improve gut health status. Using enzymes

or enzyme-degradation products has been shown to be effective to increase butyrate-producing bacteria and butyrate concentrations in the ceca. Elevated butyrate concentrations in the gut can in turn increase the density of L-cells in the GIT. The L-cells produce GLP-2, which is recognized to have potent growth-promoting activities on intestinal epithelium and GLP-1, which has important actions on gastric motility and on the promotion of satiety. The use of Enz is also often associated with increased villi length and a reduced T-cells infiltration in the GIT and by consequent less inflammation and better intestinal health status that leads to better absorption and use of nutrients. The interaction between the microbiota and the host are suggested to be the key effect of prebiotic-like components released by enzymatic degradation. The profiling of the microbiota along the gut showed that microbial diversity is still very low in the first days post-hatch. This period is likely the best one to target to get an influence on microbiota that could last till the end of the production cycle. Moreover, Enz supplementation reduces performance variations between the animals, which results in a more cost-effective production. In conclusion, when considering the effects of enzymes on animal performance it is important to take into account their effects on gut health.

Key Words: enzyme, gut health, prebiotic, NSP

598S Net energy: The most valuable concept to address the full benefit of enzyme supplementation in feeds for monogastric animals. J. Noblet*[1], S. WU[2], R. A. Swick[2], and M. Choct[2], *[1]INRA, Rennes, France, [2]University of New England, Armidale, Australia.*

Feed is the most costly component of monogastric livestock production (>60%) and energy represents the largest cost factor in the feed (>70%). The chemical energy (or gross energy: GE) of a feed or of nutrients composing the feed organic matter can be only partly retained in the body of a growing animal or in the milk of a lactating animal or in the eggs of a laying hen; the remainder is lost as chemical energy in the feces, fermentation gases, and urine and as thermal energy (i.e., heat) to the animal's environment. The heat is composed of a basal and animal related fraction (Fasting heat production, FHP) and a fraction directly dependent on the quantity and nutrient composition of feed energy (Heat increment, HI). The ways and the steps the productive (growth, milk, egg, etc.) animals use energy are then represented in different energy systems, namely, digestible energy (DE; GE minus fecal energy), metabolizable energy (ME; DE minus urine and gas energy) and net energy (NE; ME minus HI), the latter representing the closest estimate of the "true" energy value of a feed or a nutrient. The objectives of this contribution are to review the most important dietary factors affecting the different steps of energy utilization in poultry and pig with more focus on the ME to NE step and the possible effects of enzymes (carbohydrases, phytase) supplementation on energy values. In both groups of monogastrics, the DE/GE or ME/GE ratios are mostly affected by the dietary fiber (DF) levels in the feed. Similarly, the NE/ME ratio which averages 75% in both groups increases with EE and decreases with dietary CP but the impact of EE and CP on NE/ME ratio would be higher in pigs than in poultry in connection with higher differences in NE/ME values between EE, starch and CP energy for pigs (85–90%, 80% and 60%, respectively) than for poultry (85–90%, 75–80% and 65–75%, respectively). These remarkable similarities between animal species for NE/ME variations have marked consequences on the relative energy values of ingredients in DE, ME, and NE systems and justify the move from DE or ME to NE systems in both pigs (already done) and poultry (not done). This move will be reinforced in the case of enzyme-supplemented diets that will affect 1/ the nutrients composition originating from digestion and 2/ the HI per unit of ME intake and then the NE/ME ratio with potential higher impacts in poultry than in pigs. This move should also help for a better control of feed efficiency in monogastric livestock production.

Key Words: net energy, poultry, pig, enzyme, feed

Symposium: Alternative Poultry Production Practices: What Are the Impacts on Management, Animal Health, Product Quality, and Food Safety?

599S American Pastured Poultry Producers Association (APPPA) perspective of alternative poultry production practices. G. Phelan*[2] and M. Rothrock[1], [1]*USDA-ARS, Athens, GA,* [2]*American Pastured Poultry Producers Association, Hughsville, PA.*

The American Pastured Poultry Producers Association (APPPA) is a nonprofit educational and networking organization dedicated to encouraging the production, processing, and marketing of poultry raised on pasture, and is the largest industry group focused on pastured poultry. APPPA passionately embraces humane, people-friendly, environmentally enhancing, pasture-based production models. Twenty years ago, when APPPA was founded, knowing how pastured poultry differed from the conventionally-raised poultry was easy. As the integrity food movement has gained momentum, the gap between commodity and craft poultry has become a confusing marketplace of labels. Pastured poultry require 2 things: access to grass (that's pasture) and frequent movement (rotation). Birds live outside as appropriate for the age of birds and the season of the year. Realizing that production models must be profitable to be successful, APPPA's interests include processing, packaging, cooking, marketing, and any other topics related to pastured poultry enterprises. APPPA's world vision is to see pastured poultry adopted as the model for environmentally, emotionally, and economically sensible poultry production. This vision includes decentralized food systems, farmstead-sized processing, and as much interaction as possible between producer and consumer. APPPA sees pastured poultry as the model for environmentally, emotionally, and economically sensible poultry production that can feed your local communities uniquely nutritious, delicious, and humanely-raised chicken, eggs, turkey, ducks, geese and guineas. For this symposium, I will discuss how pastured poultry is different from traditional, conventional-scale poultry production, and how we see these management differences effect animal and environmental health, product quality and product safety.

Key Words: pastured poultry, animal health, food safety, product quality

600S A sneak peek into the "alternative" poultry farming systems around the globe. A. Morey*, *Auburn University, Auburn, AL.*

Alternative poultry farming systems such as small flock, backyard, free-range and cage-free are being increasingly adopted by both urban and rural poultry farmers and enthusiasts in the US. These "alternative" systems, although trendy in the US, are mainstream poultry farming practices in under-developed and developing countries around the globe. The "alternative" systems serve as a major source of income and nutrition to several people around the globe. Small-scale chicken rearing systems have a flock size anywhere from 2 to a few hundred and is seen as a low-effort, low-cost operation, with a small footprint. Poultry farmers buy chicks from local resources, Government-run hatcheries at a subsidized rates or the chicks are donated to targeted families under assistance programs. Chicks are often raised in yards with the nutrition obtained from scavenging and household waste. Some economically stable backyard farmers provide supplemental, balanced feed to increase production. Chickens are often raised as part of an integrated agriculture operation for example, poultry houses built above aquaculture ponds. Chickens raised in these alternative systems lack veterinary oversight and are at a constant risk of disease and parasite infections. Innovative approaches using social media and smartphones has helped some Governments to keep track of diseases remotely. Ultimately, the eggs and meat produced are either consumed by the family or sold in the local markets at a much higher price than regular broilers. Farmers with a few hundred chickens usually sell a small number of chickens to a local butcher on a regular basis thus creating a steady stream of income. Countries around the globe have made significant strides in the "alternative" poultry systems. Poultry veterinarians have been successful in developing strains of dual-purpose birds which are tolerant to temperature stresses. Governments have dedicated resources to develop and promote poultry farming to kindle entrepreneurial spirit among the rural population as well as a means for socioeconomic and nutritional upliftment. Efforts are being made to encourage women to take up poultry farming as a way to support their families. In some parts of the world, poultry is seen as a method to keep rural youth from conducting illegal activities. Global advances in the "alternative" poultry farming systems can be adopted to improve both, the alternative and commercial, poultry farming systems in the US.

Key Words: alternative poultry system, integrated system, backyard, free-range, cage-free

601S Management, nutrition, and animal well-being in small-scale "pastured" poultry production. A. Fanatico*, *Appalachian State University, Boone, NC.*

Small- and medium-scale alternative poultry production can have ecological and social benefits, and provides products for local markets. The number of small farms has recently begun to increase in the US, and many producers raise small batches of poultry. Extensive production systems are often used with moveable housing and electronet fencing to make portable yards. Shelters or pens are commonly used when raising meat birds during warm months. Fresh forage can provide protein, vitamins, and phytochemicals to birds. Poultry often do not take advantage of open pasture, but yard enrichments can encourage them to forage. Many small producers use fast-growing genetics for maximum yield; however, alternative genetics, such as slow-growing or medium-growing birds, may be better adapted for foraging extensive areas. Foraging activity results in the spread of manure gradually over extensive pasture, improving fertility. Small farms are usually diverse and can grow some of their own feed crops. Alternative feeding methods can utilize these farm-raised feeds and increase nutrient cycling and sustainability on small farms. Small-scale poultry production has impacts on animal well-being, biosecurity, and food safety. Poultry have the opportunity to forage and express natural behaviors in open yards, although predators may be a concern. If minimal housing is used, birds may not be adequately protected from the elements. Biosecurity practices can help reduce the introduction of disease for small flocks raised without pharmaceuticals. Chickens and turkeys are often processed on-farm under federal exemptions and sold to local markets. Because many diversified farms have crop, vegetable, fruit, and livestock operations, food safety management is important to reduce risks for poultry as well as fresh produce on the farm. When critically analyzing land use, resource use, and income efficiency, raising poultry with extensive outdoor access is generally less efficient in terms of performance than indoor production, because free-range meat poultry take longer to grow and require more feed. However, in sustainable production, maximum yield and profit are not the only goals; environmental and social impacts are also important. Extensive poultry can be integrated in complementary production

systems with grazing animals and agroforestry and provide ecosystem services. Pastured poultry production also has many applications in international development. While small-scale, ecological chicken meat production has environmental and social benefits and contributes to local food systems, it is important to ensure biosecurity and food safety.

Key Words: meat chicken, free-range, small-scale, extensive, food safety

602S Laying hen management and well-being in extensive housing systems. D. Karcher*, *Purdue University, West Lafayette, IN.*

Laying hen housing systems are moving from intensive to extensive housing systems. The extensive housing systems have been used by smaller flock sizes and niche markets resulting in eggs produced in organic, free-range, pasture or cage-free systems. Each of these different environments have similar but unique management practices that need to be employed to maximize production and hen well-being. These practices may revolve around nutritional strategies, bird thermal comfort, stress mitigation approaches and hen welfare. A better understanding of the intersection of these practices with hen welfare can result in an overall improvement in hen well-being in these extensive housing systems.

Key Words: well-being, hen housing, extensive systems

603S Application of pre/probiotics to improve animal health and reduce foodborne pathogens in pastured poultry. S. Ricke*, *University of Arkansas, Fayetteville, AR.*

Alternative poultry production systems such as pastured broiler flock operations must contend with potential food safety issues during the entire production cycle. Exposure to foodborne pathogens represents an ongoing challenge due to the wide range of potential sources that these organisms inhabit. Feed additives that have the potential to limit foodborne pathogen establishment in pasture flock birds is somewhat limited due to the restrictions of the production system. Over the past few years several biological feed additives have been examined for their potential efficacy in pasture flock birds. Probiotics and prebiotics have been studied as dietary feed amendments for not only limiting foodborne pathogen colonization but promoting overall bird health. Probiotics are cultures consisting of either single or multiple viable microorganisms possessing the capability of becoming established in the gastrointestinal tract (GIT) and impacting the GIT for a detectable benefit to the host. Probiotic cultures can also serve as a barrier to prevent the colonization of foodborne pathogens such as Salmonella in the bird GIT by several potential mechanisms including the production of short chain fatty acids and competitive exclusion. Prebiotics are defined as dietary compounds that selectively enhance beneficial indigenous GIT bacteria such as Bifidobacterium species and lactic acid bacteria already present in the GIT. While GIT microorganisms can use these prebiotic compounds as substrates for growth, the bird cannot use these compounds because they are resistant to degradation by host digestive enzymes. Once these beneficial GIT indigenous bacteria become more prominent they can inhibit foodborne pathogen establishment through production of antagonistic fermentation products as well as other metabolic activities. Historically, the most commonly used prebiotics were specific oligosaccharides including fructooligosaccharides (FOS) and related carbohydrate polymers. More recently, the concept of what constitutes a prebiotic has been broadened to encompass other carbohydrates such as certain fiber components that elicit "prebiotic-like" properties. Consequently, there is increasing interest in novel sources of prebiotics such as cereal grain brans that can be supplemented in diets. This presentation will discuss currently utilized prebiotics and application of potentially new sources of prebiotics that could be fed to pasture flock birds.

Key Words: pasture flock birds, probiotics, prebiotics, foodborne pathogens, gastrointestinal tract

604S Foodborne pathogen ecology throughout the pastured poultry farm-to-fork continuum. M. Rothrock*, *USDA-ARS, Athens, GA.*

There has been an increased demand for non-conventional raised poultry in the US, with these types of "alternative" poultry products accounting for upwards a 20% of all poultry products sold in the US. While this consumer-driven shift has resulted in this increase of alternative production, there is limited scientific data regarding any potential food safety-related issues within these systems. Therefore, the goal of this study was to look at major foodborne bacterial pathogens (*Salmonella, Campylobacter, Listeria*) and an indicator (*E. coli*) throughout the farm-to-fork continuum to see how different management or environmental/physiochemical variables effect the ecology of these bacteria. To achieve this, live production (feces, pasture soil), processing (ceca, whole carcass rinses) and final product (whole carcass rinses) samples were collected from 42 flocks from 11 pastured poultry farms from 2014 – 2017. The four foodborne pathogens were either quantified and recovered (*Campylobacter*, *E. coli*) and enriched for (*Salmonella, Listeria*) using traditionally used culture techniques, and select isolates were further characterized via speciation, subtyping, antimicrobial sensitivity testing (using the CDC NARMS panels). In general, *Campylobacter* and *E. coli* numbers were highest during live production, and greatly decreased during processing through to the final product. In terms of *Salmonella* and *Listeria*, the recovered isolated came from all sample types, but a majority of both were not characterized as posing significant threats to human health (predominantly serotype Kentucky for *Salmonella*, and *L. innocua* or *L. welshimeri*). In terms of antibiotic resistance of the bacteria from these antibiotic-free management systems, the four bacteria demonstrated different levels of resistance, with some of these differences being based on farm of origin. The results from this large study highlight to importance of continued food safety work to understand the ecological dynamics of these pathogens within this growing poultry management system.

Key Words: pastured poultry, foodborne pathogens, farm-to-fork

605S Food safety and product quality in extensive layer housing systems. D. Jones*, *US National Poultry Research Center, USDA ARS, Athens, GA.*

Throughout the world, there is a wide array of commercial and custom laying hen housing systems. Most are more extensive designs than conventional cages and provide designated spaces for egg laying, feeding, foraging, and perching. Each unique variation of laying hen housing presents opportunities and challenges for ensuring egg safety and quality. Management of a flock, regardless of the housing system, impacts egg safety and quality. Hen access to litter and outdoors has been shown to affect product safety and quality. The design of nest boxes in extensive housing systems cannot only influence laying hen preference for use, but also the ability of hens to interact with eggs after oviposition. Furthermore, egg handling and storage is a major contributor to the overall safety and quality of eggs produced in any housing system. Understanding the influences on egg safety and quality pertaining to extensive layer housing systems allows for informed management decisions.

Key Words: egg safety, egg quality, hen housing, extensive systems

606S Meat quality from alternatively raised broilers. C. Alvarado*, *Texas A&M University, College Station, TX.*

Consumers in the United States (US) are increasingly interested in poultry meat from alternatively raised broilers including but not limited to free-range, antibiotic free, GMO free, vegan fed, organic, and other new production systems being researched. Consumers who purchase chicken meat from alternatively raised practices are not as concerned with cost of the meat as compared with other consumers. However, process characteristics such as production methods, animal welfare, processing conditions, perception of health benefit, support of local communities, and environmental systems have all become increasingly important when discussing purchasing power of consumers interested in these alternative systems when compared with traditional commercial practices. With the increased availability of poultry meat from these alternative systems, several studies have focused on the impact on meat quality and consumer perception as related to sensory attributes. Research has indicated that these alternative rearing systems have many similarities regarding meat quality and sensory attributes when compared with commercial production practices. Some studies have also indicated similarities in breast meat while dark meat such as thighs and drums from birds raised using the alternative systems had a stronger attachment to the bone. A recent study indicated that trained sensory panelists were able to discriminate chicken breasts from different production sources, whereas untrained consumers were not able to discriminate. However, in this same study, consumer likeability was affected by the information provided to the consumers on the type of broiler production system. Composition of poultry meat was not very different when compared with the conventional system, but these results were variable and depended upon nutritional sources for the birds. Meat from some of the alternative systems such as free-range poultry had a shorter shelf life than meat from commercially raised birds, however, these differences can be related to processing systems as well. Therefore, meat quality from alternative production systems when compared with conventional systems is similar in many parameters; however, this can be variable as there are so many factors during production, processing, storage, and cooking which can impact meat quality and acceptability. Regardless, these alternative systems are growing in popularity and demand and more research should be conducted to determine impacts on consumer preference, availability, and meat quality.

Key Words: alternative production, meat quality, yield, broilers, free-range

Symposium: Informing the Future Using the Poultry of the Past

607S North American breeds: Potential for new markets—what's at risk? A. Martin*[1], D. P. Sponenberg[2], J. Beranger[1], and C. Couch[1], *[1]The Livestock Conservancy, Pittsboro, NC, [2]Virginia Tech, Blacksburg, VA.*

Biodiversity is a reservoir of genetic variation. Populations subject to little intensive selection for production traits, or to high levels of natural selection, are likely to retain more variation for adaptive traits. Such variation is important to conserve for unpredictable future needs. Likewise, as genomic tools become ever more powerful, diverse populations are likely to provide important allelic variants for production traits. Among North American poultry, conservation of biodiversity is most readily accomplished at the population or breed level, including commercial populations, research lines, standard breeds, landraces, and heritage breeds. Such populations are maintained by numerous institutions, in situ and through cryopreservation. Nevertheless, there has been a contraction in research populations held by public institutions, numerous heritage breeds remain at risk, and even commercial populations have contracted due to changing economies and corporate consolidation. Cost remains a major hurdle for in situ support, and emerging cryopreservation techniques for poultry must be further developed to mitigate this cost. New models to better maintain diverse populations are needed, otherwise many unique lines, and genes, will be lost. One new opportunity for maintaining diversity is emerging through the growth of niche markets for pastured poultry products. Fulfillment of this market using standard or heritage breeds alongside new broiler hybrids adds an important means for preserving genetic diversity while meeting consumer demand.

Key Words: genetics, breed, biodiversity, conservation, market

608S Genetic relationships among conservation populations of chickens. M. Berres*[1], J. Kantanen[2], M. Honkatukia[2], and J. Fulton[3], *[1]UW-Madison, Madison, WI, [2]Natural Resources Institute Finland (Luke), Helsinki, Finland, [3]Hy-Line International, Dallas Center, IA.*

Conservation of genetic diversity in agriculturally important species is required for sustainable agriculture. Climate change, newly emergent disease, increased pressure on land and water resources, and shifting market demands require that animal genetic resources are conserved and used sustainably. In commercial layer and broiler chickens, genetic variation is often maintained passively within and among limited numbers of breeds, strains, and lines. Modulated by continued selection for desirable production-level traits, there is compelling evidence indicating that commercial lines have reduced genetic variation compared with their ancestral progenitors, Red Junglefowl. Extant Junglefowl species, including heritage breeds of poultry, contain reservoirs of untapped genetic variation. Heritage breeds have experienced reduced levels of artificial selection but still have traits allowing them to adapt to differing local environments, agricultural practices, and cultural conditions, which may be quite different from those encountered in modern agriculture. Building knowledge of these existing genetic resources requires proper genetic inventories of available genetic assets. A PlexSeq (Agriplex Genomics) SNP panel consisting of 101 loci, with at least one locus present on 26 of the 38 (68%) chicken autosomes, generated genotypes that were used to determine the magnitude of genetic diversity and population structure in: (1) heritage Finnish Landrace chickens; (2) heritage broilers from Canada and the United States, and (3) standard breeds of chickens within Canada. Particularly evident in the Finnish Landrace, analysis of molecular variance (AMOVA) revealed substantial SNP variation at the among-population level and reduced variation among individuals within a population. STRUCTURE-based analyses indicated significant population-level stratification among the Finnish Landrace chickens and most heritage broilers and standard breeds from Canadian and US sources. Historical relationships were identifiable and even source populations of synthetic lines could be identified based on admixture proportions. Once thought applicable only to rare and endangered species, themes of conservation biology and population genetics combined with next-generation genetic technologies are now providing unprecedented opportunities to define and ultimately conserve genetic variation in wild, heritage, and commercial poultry. The potential to augment commercial poultry genetic management is substantial and both wild junglefowl and heritage breeds should be considered an invaluable genetic reservoir to protect and used to help maintain a healthy poultry industry.

Key Words: conservation, genetic diversity, population structure, SNP, heritage breed

609S MHC-B variation in global chicken populations. J. Fulton*, *Hy-Line International, Dallas Center, IA.*

The major histocompatibility complex (MHC) of the chicken consists of 2 clusters of highly polymorphic immune response genes, MHC-B and MHC-Y, both of which are located on chromosome 16 but which segregate independently. The chicken MHC-B was initially identified as the B blood group locus. Multiple studies have shown a strong association between variation within the MHC-B region and resistance to numerous disease pathogens. MHC-B variation can be identified utilizing a panel of single nucleotide polymorphisms (SNP) that encompass 230,000bp of the MHC-B region. The specific combination of SNP alleles across this region defines a haplotype. The MHC-B haplotypes were studied within a diverse set of populations including traditional breeds in Germany, the Finnish Landrace breed, heritage breeds within the US and Canada, and rare breeds within the US. A high amount of MHC-B variation was found among these populations. MHC-B haplotypes from common breeds could be identified in some of the populations, suggesting historical introgression from these breeds. On average, 50% of the haplotypes within a breed were novel, with 85% of these being breed specific. The rare or endangered breeds within the US appeared to have suffered an MHC bottleneck as the traditional breeds sampled in Germany had an average of 5.1 vs 3.3 hapotypes found in the US sampled breeds. These traditional or rare breeds are a source of untested novel MHC variation. With the deceasing availability of therapeutics for disease treatments in poultry, novel MHC variation should be examined in the context of better understanding of disease resistance.

Key Words: MHC, diversity, chicken, heritage breed

610S Poultry models informing human health science. C. Ashwell*, *North CarolinaState University, Raleigh, NC.*

Poultry species have fed humans for many centuries. With domestication and selective breeding poultry will continue to be a major protein source for a growing global population. Beyond their agricultural significance, poultry species have made numerous contributions to human health. Likely the first vertebrate to be studied developmentally due to the innovation of artificial incubation, the chicken embryo was the basis

of all developmental biology before the genomic era. Notable scientists including Pasteur, Rous, and Varmus have utilized the chicken and its viruses to advance the understanding of biology. Landmark discoveries in immunology, nutrition, virology, endocrinology, oncogenesis, angiogenesis, and genetics have been and will continue to be conducted in poultry species. These scientific milestones and new pioneering research will be discussed in light of significant advances in technologies including stem cells and genome editing.

Key Words: poultry, human health

611S Animal resources—Inventory, unique features and challenges. N. Anthony* and S. Orlowski, *University of Arkansas, Fayetteville, AR.*

Selection programs in university settings are dying across the US and Canada. There are many factors that contribute to this loss with the most obvious being financial and infrastructure costs. In addition, university, departmental and personal challenges are faced every day by researchers maintaining experimental lines. Although efforts have been made to indefinitely preserve genetic material they continue to be unreliable for long-term storage. Resource populations maintain genetic variation that would have otherwise been lost. The development of random bred control populations have allowed for a snapshot of the poultry industry in 20 year increments starting with the Athens Canadian Random bred in 1955 and ending with the development of the most recent random bred control in 2015. These lines have been instrumental in evaluating the maturation of the poultry industry. Random bred populations have served as base populations and respective controls for selection studies designed to explore direct and correlated responses as well as dissect metabolic challenges that crop up as a result of traditional selection practices. Other resource populations have focused traits that have been identified, preserved and concentrated through selection. Many of these populations serve as research models to study human disease conditions. The expectation is that these research lines would dovetail with the basic and applied programs of colleagues and collaborators. Although granting agencies typically do not support the development of research lines, the inclusion of research lines in a grant proposal clearly elevates the overall proposal success. The purpose of this presentation is to provide an overview of the research lines developed over the past 30 years as well as a discussion of the challenges faced by those maintaining experimental populations.

Key Words: broiler, genetic preservation, research lines, random bred control

612S Grassroots support for genetic preservation programs. M. Zuidhof*[1] and V. Carney[2], [1]*University of Alberta, Edmonton, AB, Canada,* [2]*Alberta Agriculture and Forestry, Edmonton, AB, Canada.*

Genetic preservation programs all over the world have been dealt a serious blow by economic realities. Most government and university reservoirs of historically relevant and scientifically valuable lines have been terminated due to budget cuts over the last 3 decades. The Poultry Research Centre (PRC) at the University of Alberta has managed to maintain 11 lines of historical and scientific importance by engaging grassroots support. Since 2014, the PRC has engaged the public in an effort to recover the cost of maintaining these valuable lines by developing 3 innovative programs. Members of the community were first invited to adopt a hen, then to eat heritage meat, and most recently in partnership with a regional agricultural supplies retailer, to consider growing heritage chicks for themselves. This initiative required considerable effort to mitigate risks, build and manage relationships, and reach out to a new demographic with the will to help save our unique genetic resources. As a result of this effort, however, the genetic preservation program is now financially self-sustaining. As an additional benefit, our Heritage Chicken Program provides unique and expanded opportunities for teaching, extension, research, and connecting with consumers.

Key Words: genetic conservation, community engagement, funding, performance benchmark

National Outreach Workshop: The Future of Agriculture-Serving Audiences of all Sizes Through Integration of Research and Outreach

613S Assessing stakeholders' needs from both extension and research perspectives. B. McCrea*, *Auburn University, Auburn, AL.*

Translating research into a form that is both understandable and usable for the poultry farmer or poultry industry personnel is one of the many roles of the extension professional. Many extension poultry specialists, and extension poultry veterinarians, also have a research or teaching appointment, occasionally both. As such, these extension professionals often translate their own research into something usable for those within their own states or regions. To prepare materials that meet the needs of their stakeholders, these extension poultry professionals took steps to understand the needs of their constituents after many conversations and consultations. Stakeholder input drives the plans of work of extension professionals. A needs assessment is often one of the first things that a new extension poultry professional performs in their position and this process can take many forms. A potential negative aspect is that it can be time consuming. However, not all needs assessment tools are the same, nor are they delivered in the same manner. Depending upon the institution, there may be specific protocols to be followed, or there may be someone within extension to help design or even disseminate the needs assessment tool. Finding stakeholders, in some cases, can be as difficult as getting input from them. The time commitment to establishing relationships with stakeholders can be significant. In addition, maintaining meaningful contact with some scattered groups can be challenging. Extension professionals have tools that they use to find and engage those who may not be familiar with the services provided through poultry extension. There is a perception that extension can be an afterthought, one that is simply "tacked-on" to the end of grants. For those reaching out to extension from the research arm of a department or college, a needs assessment may not have been previously considered. When writing grants, it is important that all writers understand the stakeholders that are to be affected by potential research and the extension that is to follow. The questions asked by the extension professional often are slightly different from those of the research professional, but are no less important. Great collaborations and excellent grant proposals are ones in which both the research and extension arms understand the needs of the stakeholder.

Key Words: extension poultry specialist, extension poultry veterinarian, stakeholder needs, producers, research

614S Engaging stakeholders. P. Curtis*, *North Carolina State University, Raleigh, NC.*

Engaging stakeholders is crucial to the success of any outreach program. There are many ways to engage stakeholders. Some are more effective than others. We all know it is very difficult to engage an audience by presenting a lecture. To truly engage with someone, we need 2-way interaction. Therefore, to engage our stakeholders we need to determine how their interest matches our interest. What I would like to share with you today is one of the approaches we are using at NC State to engage our stakeholders. When we approach our stakeholders, we begin our discussions talking about a partnership between the stakeholder and the university. The Prestage Department of Poultry Science has spent the last year creating a vision and then a strategic plan which is built on partnerships for achieving goals. We have involved our stakeholders in each step of the process. Our partnership discussions require truly engaged stakeholders to be successful. We start by asking our stakeholder for their vision of the future and then share our vision. We begin the engagement by discussing the overlaps in our visions and asking the stakeholder if there are specific areas of overlap where they would like to partner with us to create a synergy that will help both of us to reach our goals. This approach has worked very successfully for us. It works for small and large projects. We have used it successfully for recruiting, sponsorships, graduate assistantships, endowed professorships and facilities.

Key Words: stakeholders, partnerships, outreach, extension, funding

615S The triathlon for success: Integrating research, outreach, and stakeholders to support poultry producers. A. Donoghue*, *ARS, USDA, Fayetteville, AR.*

Research scientists, extension agents, poultry industry farmers/leadership are focused on producing high quality, safe protein sources for consumption. Although areas of focus differ a bit for these individual areas of expertise, a powerful synergistic approach can result in highly productive teams to resolve important issues. As many of the granting programs require integrated approaches (e.g., NIFA Organic Research and Extension Initiative (OREI) Program or Sustainable Agriculture Research and Education (SARE) grants), there are incentives for these groups to work together. Although this appears to be logical, effort has to be taken to create functional teams. Many of us have experienced receiving feedback from grant panels or have served on grant review panels that result in comments suggesting the integration does not translate for a particular proposal. Sometimes these thoughts are reflected in comments such as – the research or extension portion appears to be tacked on to the proposal and not truly integrated; evidence of true stakeholder integration is unclear in the statement of work. Developing successful integrated teams can be achieved by utilizing and recognizing the strengths each expert brings to the project and utilizing this expertise. Several elements have helped our teams develop truly integrated projects. First, relationships with farmers and members of the industry that are active in seeking results to issues is key. Some of our best ideas have been generated by farmers who have challenged our team to help them find a solution. Second, recognizing that researchers sometimes do not know the depth of how extension works and vice versa. Those focused on fundamental research may believe they are qualified to write the extension/outreach portions of a project without truly relying on those who perform these activities as a major part of their job. Farmers can be eloquent in verbalizing an issue or a potential strategy but do not have the time or practice in putting these ideas on paper. Additionally, working across institutions can make powerful teams yet understanding the timeline requirements for the grant support offices is important to make deadlines. Further, Agricultural Research Service (ARS) scientists can make important contributions to proposals as their focus is long-term and potentially large-scale research adding unique opportunities to these collaborations. Working across disciplines lends itself to collegial interaction and holistic approaches to solving critical and emerging issues for our poultry industry.

Key Words: stakeholders, integrated projects, research, teaching, outreach

616S Balancing small flock extension with research planning: Resources and strategies. B. McCrea*[1], J. Moyle[2], A. Fanatico[3], T. Lavergne[4], and J. Jacob[5], *[1]Auburn University, Auburn, AL, [2]University of Maryland, Salisbury, MD, [3]Appalachian State University, Boone, NC, [4]Arm & Hammer Animal Nutrition, Baton Rouge, LA, [5]University of Kentucky, Lexington, KY.*

Extension to small flock stakeholders can be challenging. There are, however, resources to aid both extension and research poultry professionals balance their efforts to provide science-based information for this burgeoning group of poultry owners and producers. This group encompasses backyard flock owners; hobbyists; small- and medium-scale producers; transitioning and organic farmers; pastured poultry producers; and several other niche producers. They are often independent in that they may not have an organization to which they belong, and are not as likely to come together for meetings. The needs of these groups may be greater given a distinct lack of information that is specifically designed for their niche markets. They are also small enough that they are not typically able to fund research projects of sufficient scale to produce meaningful results. In addition, there are not many funding opportunities available through grants even though the impact of research is likely to reach a large number of farms, birds, flock owners, and entrepreneurs. It is difficult for new extension poultry specialists and veterinarians to find the right balance of working with both small flock owners and commercial poultry producers. Small flock owners can demand a great deal of time and yield high returns on knowledge gained. The challenge for our poultry professionals is in getting producers to adopt practices stemming from science-based research that has been specifically designed for niche management systems. This panel will provide an opportunity to discuss different strategies in reaching small flocks, deliver information that is likely to be implemented, and balancing these efforts with needs of commercial producers as well as research programs.

Key Words: small flock, niche market, organic, backyard, pastured

Author Index

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