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SYMPOSIA AND ORAL PRESENTATIONS

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Student Competition: Metabolism and Nutrition, Feed Additives I

1 Early probiotic application improves hatchability and hatchling quality of broiler chicks. Mairui Gao^{1*}, Yuying Ren¹, Ragini Reddyvari¹, Si Lu¹, Mary Anne Amalaradjou¹, ¹*University of Connecticut, Storrs, Connecticut, United States.*

A broiler's life includes pre-hatch incubation and post-hatch grow out. Post-hatch performance is directly associated with the yield of carcass, thus attracting significant research interests. However, the pre-hatch period (~3 weeks) takes up one-third of a broiler's whole life. Specifically, optimum embryonic development can exert significant influence on chick hatchability and hatchling quality which in turn can affect subsequent broiler growth and performance. As with a higher hatchability, healthy and active hatchlings are also correlated with improved post-hatch performance. Therefore, we evaluated the effect of in-ovo probiotic spray application on broiler hatchability and hatchling quality. Supplementation of probiotics to the growing embryo improves hatchability and hatchling quality of broiler chicks. A total of 1080 Ross 308 eggs were obtained from Aviagen for the study. The experiment was set out as a completely randomized study with two independent trials. In each trial, eggs were sprayed with 200 µl of phosphate buffered saline (PBS; control) or probiotics [~8 log CFU/egg of *Lactobacillus rhamnosus* NRRL B-442(LR) or *Lactobacillus paracasei* DUP 13076 (LP)] on D0, D3, D7, D10, D14 and D18 of incubation. Hatchability was calculated at D21.5. Hatched chicks were scored for their quality using the Tona and Pasgar score. Additionally, morphometric measurements including hatchling weight and length were recorded at necropsy. Data were analyzed using GraphPad Prism 9.3.0, and $p < 0.05$ was considered as significant. Overall, in-ovo probiotic supplementation improved hatchability and hatchling quality. Specifically, the application of LP improved hatchability by ~5% when compared to the control. Further, both LR and LP significantly ($p < 0.05$) improved hatchling quality. Particularly, hatchlings in the probiotic treated groups were found to have better reflex activity, and appearance, indicating an improved hatchling quality. Moreover, probiotic treated groups tended to have a significantly higher body weight at hatch when compared to the control group ($p < 0.05$). This is significant since chick weight at hatch is considered to be an indicator of post-hatch performance. Hence, the above-mentioned probiotics could be employed to promote hatchability and hatchling quality. Early supplementation of probiotics to growing embryos potentially can be employed to promote hatchability and hatchling quality. Further, it could be used in conjunction with current post-hatch approaches to improve overall growth and performance of broiler chickens.

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Key Words: broiler; hatchability; hatchling quality; early probiotic supplementation

2 Growth performance and organ weights of broilers given an *Eimeria* challenge with or without a novel *Bacillus subtilis* probiotic treatment. Alison Gelinas^{1*}, Sudhanshu Sudan¹, Julang Li¹, David Huyben¹, Elijah Kiarie¹, ¹*University of Guelph, Guelph, Ontario, Canada.*

Bacillus subtilis-based probiotics are recognized alternatives to antibiotic growth promoters in poultry production. We examined the efficacy of a novel *B. subtilis* (BS-9) (PRO) isolated from camel dung to evaluate growth performance and organ weights in broiler chickens challenged with *Eimeria*. Ross 708 d-old male broiler chicks were placed in 28-floor pens (25 birds/pen) and allocated to two water treatments (trt), control or PRO (10^8 CFU/mL of water) (n=14). Water was provided in bell drinkers and treated daily with a fresh culture until d 13 of life. Birds had *ad libitum* access to a balanced corn/ soybean meal-based diet. On d 9, all birds in half replicate pens (n=7) received 1 mL *E. maxima* and *E. acervulina* mixture via oral gavage, and birds in the other half of replicate pens (n=7) received a sham. Bodyweight (BW), feed intake (FI), and mortality were recorded on d 0, 9, 14, 19, 28, and 35 for assessing pre- and post-challenge growth performance. A total of 4 birds/ pen and 2 birds/pen were necropsied for organs weight on d14 and 35, respectively. The data was subjected to GLMM procedures of SAS with trt as a fixed factor for the pre-challenge (d 0-9) and trt, challenge, and associated interactions as fixed factors for the post-challenge period (d 10-35). There was no trt effect on pre-challenge growth performance. In the post-challenged period, there was no interaction ($P < 0.05$) between trt and challenge on d 35 BW and BW gain (BWG). The main effects were such that *Eimeria* reduced ($P < 0.01$) (1,774 vs. 1,911 g/bird) whereas PRO increased (1,875 vs. 1,811 g/bird) d 35 BW. There was an interaction between trt and challenge on FI ($P = 0.021$) and mortality corrected feed conversion ratio (FCR) ($P = 0.022$). PRO birds had a higher FI than challenged PRO birds and all control birds. *Eimeria* challenged control birds had poorer FCR than unchallenged control birds and PRO birds, however, the FCR of PRO birds with and without challenge was similar. *Eimeria*-challenged birds had heavier d 14 liver ($P < 0.01$) and d 35 spleen ($P = 0.01$). An interaction ($P = 0.04$) between trt and challenge on d 14 bursa weight was such that among the PRO birds, the bursa of *Eimeria* challenged birds were heavier than non-challenged birds. Results indicated that *Bacillus subtilis* (BS-9) supplementation improved broiler growth performance during an *Eimeria* challenge, possibly through immunological modulation as reflected by the bursa weight.

Key Words: broilers; *Bacillus subtilis*; coccidiosis; growth performance; probiotic

3 Evaluation of propolis as feed supplement against *Salmonella* infection in broiler. Hanseo Ko^{1*}, Janghan Choi¹, Doyun Goo¹, Milan Sharma¹, Dima White¹, Woo Kim¹, ¹*University of Georgia, Athens, Georgia, United States.*

The objective of this study was to evaluate the effect of dietary propolis supplementation on growth performance and *Salmonella* colonization in broilers challenged with *Salmonella Typhimurium* (ST). A total of 637 one-day-old male Cobb 500 broilers were randomly allocated into 7 treatments with 7 replicates and 13 birds per cage for 21 d. The treatments included non-challenge control (NC) and ST challenge groups with different dietary propolis supplementations levels (0, 50, 100, 150, 200, and 250 mg/kg diet). On d 0, all birds in ST challenge groups and NC group were orally challenged with ST (0.5 ml of 1.3×10^8 CFU/ml per bird) and PBS (0.5 ml per bird), respectively. Body weight (BW), BW gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were measured weekly. For calculating mortality, the number of dead birds was counted daily. ST loads in ceca and incidences of ST positive samples (liver and spleen) were tested at 4, 7, 14, and 21 d. One-way ANOVA with Tukey's post-hoc test was used for all data analyses except for incidences of ST positive samples, and the polynomial contrast was used in ST challenge groups for evaluating the propolis dose effect. A chi-squared test was used for incidences of ST positive samples. Quadratic effects were observed in BWG ($P = 0.002$) and FI ($P = 0.001$) during 0 - 7 d, and the BWG of the 100 mg/kg propolis treatment was greater ($P < 0.001$) than that of the ST challenge control (0 mg/kg propolis). An increase in dietary propolis showed improvements in BWG and FCR during 7 - 21 d (linear, $P < 0.001$). The overall mortality was quadratically influenced ($P = 0.023$) by dietary propolis and lower ($P > 0.05$) in the 100 and 150 mg/kg propolis groups by 9.89% as compared to the ST challenge control. An increase in dietary propolis linearly reduced ST loads in the ceca at 4 and 14 d ($P = 0.004$ and $P = 0.001$, respectively). ST positive spleen samples were not observed ($P = 0.049$) in the 150, 200, and 250 mg/kg propolis treatments at d 14. In addition, ST-positive spleen samples were not observed ($P = 0.033$) in the 100, 150, 200, and 250 mg/kg propolis treatments at d 21. In conclusion, dietary propolis had dose-dependent effects on growth performance and *Salmonella* infection. However, over 150 mg/kg diet of dietary propolis suppressed the BWG and FI. Therefore, it is recommended that a lower level of dietary propolis is appropriate for broilers.

Key Words: *Salmonella*; Broiler; Antibiotic alternative; Feed supplement; Propolis

4 Dietary herbaceous mixture improves intestinal health in post-peak laying hens through enhancing intestinal barrier function and modifying gut microbiota composition. Yilu Wang^{1*}, Yao Zhu¹, Bin Wei¹, H.Y Zhang¹, Y.Q. Huang¹, W Chen¹, ¹*Institute of Animal Science and Technology, Henan Agricultural University, Zhengzhou, China.*

The intestine of Laying hens is susceptible to various adverse factors resulting in diarrhea and intestinal diseases. Some compounds in herbaceous plants have been reported to produce a positive impact on intestine health. Therefore, the current study was conducted to investigate the effect of the herbaceous mixture (HM), which consists of *Andrographis paniculate*, *Silybum marianum*, *Azadirachta Indica*, and *Ocimum basilicum* (2:3.5:1:2), on gut health in post-peak laying hens. A total of 240 Hy-line Brown hens (389-d-old) were randomly fed the basal diet with 0 mg/kg choline chloride (negative control, NC), 1,000 mg/kg choline chloride (control, Ctrl), or 300 mg/kg HM. On d 28, 8 birds (1 chicken per replication) were randomly selected, and blood was collected for lipopolysaccharide (LPS), diamine oxidase (DAO) and D-lactic acid (D-LA) determination, then these birds were slaughtered, intestinal length, weight, as well as the pH of chyme was measured immediately. The ileal tissue was collected for the analysis of LPS, secretory immunoglobulin A (sIgA) content, and expression of intestinal barrier-related protein genes. Cecum contents were gain for microbiota analysis. Data were analyzed by one-way analysis of variance (ANOVA) and Tukey multiple comparisons. The results showed that the addition of HM to the diet had no significant effect on the weight, length and pH of the duodenum, jejunum, ileum and cecum of layer hens ($P > 0.05$). The level of LPS in HM and Ctrl groups was higher than that in NC group ($P < 0.001$). The serum content of LPS, DAO and D-LA were significantly decreased by the HM supplementation as compared to NC group, and the addition of HM significantly upregulated the expression of *Mucin-2*, *Occludin* and *Claudin-1* when compared with the Ctrl and NC groups ($P < 0.05$). The outcomes of 16S rRNA sequencing showed that the addition of HM significantly improved the alpha diversity, evidenced by higher Sob, Ace index, and Chao index ($P < 0.05$) in HM birds compared with those fed the Ctrl and NC diets. Compared to NC group, dietary HM significantly reduces the relative abundance of Actinobacteria in the phylum level ($P = 0.0498$), and notably significantly increased *Clostridia* in the class level ($P = 0.044$). Collectively, the above results suggested that the supplementation of HM improves intestinal health through enhancing intestinal barrier function and modifying gut microbiota composition in post-peak laying hens.

Key Words: laying hens; intestinal health; herbaceous mixture

Student Competition: Immunology, Health and Disease I

5 Impact of live coccidiosis vaccination on ileum and cecal microbial diversity in turkeys. Maria Trujillo Peralta^{1*}, Danielle Graham¹, Jianmin Chai², Jiangchao Zhao¹, Roberto Cuesta¹, Aaron Forga¹, Makenly Coles¹, Juan Latorre Cardenas¹, Guillermo Tellez-Isaias¹, Billy Hargis¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Foshan University, Foshan, China.

Although live coccidiosis vaccines have been shown to prevent coccidiosis in commercial turkeys, the effect of vaccination, with and without a bioshuttle program, on the gut microbiome has been largely understudied. The purpose of the present study was to assess the effect live coccidiosis vaccination, with and without amprolium intervention, on the ileal and cecal microbiome 6 days post-challenge. Treatment groups included the following: 1) non-challenged, non-vaccinated control (NC); 2) challenged, non-vaccinated control (PC); 3) *E. meleagritidis* vaccine candidate isolated from wild turkey feces (VX) + amprolium contacts; 4) VX + amprolium directs; 5) VX contacts; 6) VX directs; 7) Commercial VX containing *E. meleagritidis* and *E. adenoides* contacts; and 8) Commercial VX directs. For the vaccinated groups, 50% of the poult (directs) were orally vaccinated at DOH with either 50 sporulated *E. meleagritidis* oocysts obtained from wild turkey feces (groups 4 and 6) or a commercially available vaccine containing *E. meleagritidis* and *E. adenoides* that was administered based off of manufacturer's recommendation (group 8). Directs were comingled with non-vaccinated (contact) poult for the duration of the study. Group 3 and 4 were treated with amprolium (0.024%) in the drinking water from d10-14. At d23, all groups (except NC) were orally challenged with 95K *E. meleagritidis* oocysts/mL/poult. At d29, or 6 days post-challenge, ileal and cecal contents were collected for 16S rRNA gene-based microbiome analysis. Our results showed that ileal and cecal content in PC group had different bacterial diversity and structure including alpha and beta diversity when compared to NC. Linear discriminant analysis Effect Size (LEfSe) was employed to identify bacterial biomarkers for each group, and we found that *Lactobacillus salivarius* (ASV2) was enriched in ileal and cecal content of PC. Compared to controls (NC and PC), the treatment groups (groups 3-8) showed distinct clusters in ileal and cecal community based on Bray-Curtis and Jaccard distances. LEfSe identified *Faecalibacterium prausnitzii* (ASV7 and ASV15) as a bacterial signature for ileal and cecal community in group 3, while *Turicibacter* (ASV4) was over represented in group 4. Moreover, *Streptococcus alactolyticus* (ASV6) was greater in group 7. In conclusion, live coccidiosis vaccination, amprolium intervention from d10-14, and/or *E. meleagritidis* challenge markedly affect both ileal and cecal microbiome.

Key Words: coccidiosis; microbiome; turkeys; vaccine; amprolium

6 Mixed coccidia challenge impacted the expression of inflammatory cytokines and tight junction proteins in broiler chickens. Robert Hernandez^{1*}, Kirk Klasing¹, Yanhong Liu¹, ¹UC Davis, Woodland, California, United States.

Coccidiosis is an economically significant disease in the poultry industry that causes nutrient malabsorption, depressed growth performance, and reduced feed efficiency. This study aimed to determine the effects of mixed coccidia challenges on the gene expression of cytokines and tight junction proteins in broiler chickens. One hundred and forty two-day-old Cobb 500 broiler chickens (N = 7) were group-housed in battery brooders and randomly assigned to one of four treatments: negative control or a challenge with 15×, 20×, or 25× the recommended coccidiosis vaccine dose. At 10 days of age, chickens in the coccidia treatment groups were orally inoculated with unattenuated *E. acervulina*, *E. maxima*, and *E. tenella* (ADVENT[®], Huvepharma Inc) in the feed. Weight gain and feed conversion were recorded before inoculation and at 5 and 10 days post inoculation (DPI). At 5 and 10 DPI, feces were collected for oocyst enumeration and plasma samples were analyzed for carotenoid concentration. Liver and small intestine samples were also collected for RT-qPCR analysis of cytokine and tight junction protein gene expression. Data were analyzed by ANOVA with a statistical model that included treatment, sampling date, and their interaction as fixed effects and pen as random effect. All coccidia challenges reduced weight gain ($P < 0.05$) and feed conversion ($P < 0.05$) from 5 to 10 DPI. At 10 DPI, coccidia challenges decreased ($P < 0.05$) plasma carotenoid levels and increased ($P < 0.05$) the number of oocysts per gram of feces. Coccidia challenges increased ($P < 0.05$) interferon-gamma expression in the jejunum and ileum at 5 DPI and in the duodenum, jejunum, and liver at 10 DPI. The expression of interleukin (IL)-6 at 5 DPI and IL-1 β at 10 DPI also increased ($P < 0.05$) in the duodenum of coccidia challenged chickens. A significant interaction of treatment and sampling date was observed ($P < 0.001$) for claudin-1 in the duodenum and jejunum, as coccidia challenges upregulated ($P < 0.01$) claudin-1 expression at 5 DPI. Coccidia challenges tended to decrease ($P < 0.055$) zonula occludens-1 expression in the jejunum at 10 DPI but did not affect occludin expression. These results indicate that inflammation may be differentially expressed along the small intestine or systemically and tight junction protein expression may become dysregulated in chickens challenged with mixed coccidia species.

Key Words: broiler; cytokine; Coccidiosis; tight junction; challenge

7 Dietary peptide specific antibodies to varying regions of IL-4 contribute to differential immune cell responses and performance outcomes during coccidiosis challenge in broilers. Krysten Fries-Craft^{1*}, Elizabeth Bobeck¹, ¹*Iowa State University, Ames, Iowa, United States.*

Interleukin (IL)-4 may contribute to *Eimeria* spp. establishment in the host during coccidiosis. Dietary egg yolk antibodies against targets like IL-4 in poultry may mitigate costly performance losses during coccidiosis. The study objective was to evaluate peptide-specific IL-4 antibodies and the effects on broiler performance and immunity during coccidiosis challenge. Four candidate peptides (P1-4) were selected based on antigenicity and location within the IL-4 molecule. Eggs were collected from hens injected with conjugated peptide (3/peptide) and separated yolks were pooled by peptide before freeze-drying. Eggs from hens injected with carrier protein only were used as a control. For 28d, 300 Ross 708 broilers were housed in floor pens (10 birds/pen) and randomly assigned to 5 diets with 14d starter and grower periods. Diets consisted of a basal diet + 2% egg yolk powder containing antibodies to 1 of 4 peptides or carrier-only control. On d14, 3 birds/diet were euthanized for blood sampling and half the remainder were inoculated with 10X Coccivac®-B52 (Merck, Kenilworth, NJ) for 10 total treatment groups. Body weight and FI were recorded weekly and blood samples were collected on 3, 7, and 14d post-inoculation (pi; 3 birds/group). Immune cell profiles in peripheral blood mononuclear cells (PBMC) were assessed by multicolor flow cytometry. Data were analyzed using the mixed procedure (SAS 9.4) with diet, *Eimeria*, and diet×*Eimeria* fixed effects ($P \leq 0.05$). No performance changes were observed prior to inoculation but all peptide antibodies increased peripheral CD3⁺CD4⁺ T helper cells 23.9-66.4% compared to control ($P < 0.0001$). In the first 7dpi, the *Eimeria* main effect reduced FI and BWG by 16.1 and 30.3%, respectively, resulting in a 30-point less efficient FCR ($P < 0.0001$). Only birds fed P4 antibody did not show grower FCR recovery and remained 16 points less efficient than their unchallenged counterparts ($P = 0.03$). On 3dpi, only challenged birds fed P1 and P3 antibodies had 24.9-35.0% PBMC CD3⁺ T cell reductions compared to unchallenged counterparts that were not observed in control-fed birds ($P < 0.0001$). Similar reductions roughly corresponding to T cell recruitment were not observed in challenged birds fed P2 antibodies. In contrast, challenged birds fed P4 antibodies showed a 41.3% CD3⁺ T cell reduction compared to unchallenged counterparts only on 14dpi when all other groups had similar T cell populations between challenged and unchallenged birds. These results suggest that dietary antibodies targeting different regions of IL-4 contributed to varying T cell responses during coccidiosis challenge, with delayed T cell recruitment in birds fed P4 antibodies potentially affecting performance recovery.

Key Words: Immunity; Broiler; Coccidiosis; Interleukin-4; Egg Yolk Antibodies

8 Graded levels of *Eimeria* linearly reduced the growth performance and delayed the egg production of Hy-Line W-36 laying hens when infected at the pre-lay stage of growth. Milan Sharma^{1*}, Dima White¹, Woo Kim¹, ¹*University of Georgia, Athens, Georgia, United States.*

An experiment was conducted to investigate the effects of graded *Eimeria* challenge on performance, body composition, intestinal health, and egg production of Hy-Line W-36 pullets and laying hens. A total of 360, 15-wk old pullets were randomly allocated into five treatment groups with six replicate cages, including a non-challenged control group. A mixed *Eimeria* species solution containing 50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* sporulated oocysts per mL was prepared and inoculated to one group as a high-dose treatment at wk 15. The 2-fold serial dilution was done to prepare the medium-high (25,000 *E. maxima*; 25,000 *E. tenella*; and 125,000 *E. acervulina*), the medium-low (12,500 *E. maxima*; 12,500 *E. tenella*; and 62,500 *E. acervulina*), and the low (6,250 *E. maxima*; 6,250 *E. tenella*; and 31,250 *E. acervulina*) dose treatments and inoculated to three remaining groups, respectively. The growth performance (GP) of pullets and daily feed intake (ADFI) were measured from 0-14 days post-infection (DPI), whereas body composition (BC) using Dual Energy X-ray Densitometry was measured on 6 DPI. Intestinal lesion scoring for *E. acervulina*, *E. maxima*, and *E. tenella* was done on 6 DPI. Hen day egg production (HDEP) was calculated from wk 19. Data for GP and BC were analyzed using one-way ANOVA, whereas the Kruskal-Wallis test was used for lesion score and split-plot in time for ADFI and HDEP. A significant level was set at $P \leq 0.05$, and means were separated using Fisher's LSD. A significant linear reduction in average body weight and gain was observed with increased *Eimeria* challenge dosage on both 6 and 14 DPI ($P < 0.0001$, $P\text{-Lin} < 0.0001$). The body composition of the pullets (bone mineral content and tissue weight) decreased linearly with an increase in inoculated dosages ($P < 0.05$). An interaction between the *Eimeria* dosages and DPI was observed for ADFI. Feed intake started to drop in challenged groups from 4 DPI and did not recover until 14 DPI. The most significant drop in feed intake was observed in 6 DPI in all the challenged groups. The intestinal lesion score was higher in the challenged groups than in control ($P < 0.05$); however, no significant differences were observed among the challenged groups. An interaction between the *Eimeria* challenge dosage and the age of the birds was observed for HDEP ($P = 0.0427$). Overall, the HDEP was lower in the challenged groups compared to the control. Once the challenged birds were recovered from coccidiosis, HDEP became similar to control only at wk 22, 3 wks later after starting to lay eggs. In conclusion, coccidiosis reduced the GP, altered the body composition and intestinal health, and delayed the egg production when infected at the pre-lay stage of pullets.

Key Words: Eimeria; Coccidiosis; Pullets; Dual Energy X-ray Densitometry; Hen day egg production

9 Effect of bacterial lipopolysaccharides (LPS) and mixed Eimeria co-infection on growth performance, intestinal integrity, and bone formation in broilers.

Venkata Sesha Reddy Choppa^{1*}, Guanchen Liu¹, Yuguo Tompkins¹, Milan Sharma¹, Woo Kim¹, ¹University of Georgia, Athens, Georgia, United States.

Eimeria infection adversely affects the growth and immune system of the birds through the changes in digestive physiology. Furthermore, Lipopolysaccharide (LPS) is a potent pro-inflammatory factor that may aid in investigating the influences of inflammation on bone formation and growth performance in broilers. This study aimed at the effect of LPS with concomitant inoculation of mixed *Eimeria* spp. on broiler's production parameters, gut permeability, and bone growth. A total of 360, one-day-old male Cobb 500 broiler birds were used. Uniformly weighed birds were randomly allocated into 6 treatments (T1-T6) with 5 replicated cages and 10 birds per cage. On day 14, except for the control group (T1), all the birds were inoculated with *Eimeria* species (12,500 *E. maxima*; 12,500 *E. tenella*; and 62,500 *E. acervulina*). LPS was injected intraperitoneally to T3 and T4 at 1 and 2mg/kg, respectively, on day 14. Additionally, T5 and T6 were injected with 1 and 2mg/kg doses at two-time points (day 14 and 18), respectively. Growth performance and daily feed intake were calculated from 0 to 12 days post-inoculation (DPI). Moreover, gut permeability (5 DPI), body composition (6 and 12 DPI), and bone growth rate (4 & 8 DPI) were measured using fluorescein isothiocyanate dextran, dual-energy X-ray absorptiometry (DEXA), and calcein injection, respectively. Lesion scoring was performed on 6 DPI. Statistical analyses (One-way ANOVA, Kruskal Wallis test, Tukey HSD, and Dunnett's method) were performed using SAS (version 9.4). Average daily feed intake, feed conversion ratio, body weight gains, and gut integrity were significantly decreased ($P < 0.05$) for the challenge groups (T2-T6) compared to the non-challenged control (T1). Moreover, there is a substantial increase in muscle percentage and decrease in fat percentage in challenged birds when compared with challenged birds on DPI 6 ($P < 0.05$), indicating *Eimeria* spp. and LPS have a profound impact on fat formation than muscle growth. Surprisingly, calcein measurements for T4, T5, and T6 had shown a significant reduction in bone growth when compared with the T1, T2, and T3 groups ($P < 0.05$). Although BMD (Bone Mineral Density), BMC (Bone Mineral Content), and muscle percentage didn't differ much during DPI 6, there is a significant decrease in the challenged birds ($P < 0.05$) compared with non-challenged birds on DPI 12, indicating increased risk for lameness incidence. In conclusion, all the challenge treatments had a significant decrease in several parameters compared to non-challenged birds, indicating worsening effects of coccidiosis and LPS on growth

performance, bone growth, and intestinal integrity in broilers.

Key Words: Eimeria; Gastrointestinal permeability; Lipopolysaccharide (LPS); Calcein; Dual Energy X-ray Absorptiometry

10 In vitro and in vivo evaluation of chlorhexidine salts as potential alternatives to potassium dichromate for Eimeria spp. oocyst preservation.

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Potassium dichromate (PDC) has been used for sporulation and preservation of *Eimeria* spp. oocysts for decades, but increasing safety and environmental concerns has led to exploration of alternate media for *in vitro* storage. Preliminary *in vitro* have proved the antimicrobial activity of chlorhexidine (CHX) salts. The optimal inhibitory concentration was determined to be 2.33mM CHX, specifically for CHX gluconate/digluconate or diacetate salts. Further *in vitro* testing indicated that CHX gluconate/digluconate had no effect on sporulated *E. maxima* (EMM6) oocyst integrity. However, CHX diacetate degraded EMM6 oocyst walls within 96h at 26°C. The objectives of the present study were 1) to evaluate the effect of CHX gluconate/digluconate as alternatives to PDC for *in vitro* preservation of EMM6 oocysts and 2) to utilize autofluorescence (AF%) as an indicator of EMM6 oocyst viability. For the assay, 0.20% (2.23mM) CHX gluconate (Durvet/VETone) and 0.20% (2.23mM) CHX digluconate (Sigma) were utilized for storage of sporulated EMM6 oocysts at 4°C for five months prior to an *in vivo* challenge in broilers to test oocyst viability and infectivity. After 5 months of storage at 4°C, AF was less than 12% for all challenge stocks prior to artificial aging. Additionally, samples of EMM6 oocysts stored in 0.20% CHX gluconate and 0.20% CHX digluconate were artificially aged (11 days at 45°C), resulting in 100% AF oocysts. Experimental groups (n=3 chickens/cage, n=3 cages/group) included: 1) non-challenged control; 2) 2.5% PDC oocysts; 3) 0.9% saline oocysts; 4) 0.20% CHX digluconate oocysts; 5) 0.20% CHX gluconate oocysts; 6) 100% AF 2.5% PDC oocysts; 7) 100% AF 0.20% CHX digluconate oocysts; 8) 100% AF 0.20% CHX gluconate oocysts; 9) 100% AF 0.9% saline stored oocysts. Chickens were orally challenged at day 9 of age with 500 sporulated EMM6 oocysts/chicken. Excreta was collected days 5-9 post-challenge to determine total oocyst output. Oocyst storage media and AF% both had a marked effect on total oocyst output at $P < 0.05$. Output from birds challenged with CHX gluconate stored oocysts were statistically similar to the output of those challenged with 2.5% PDC stored oocysts at 4.46×10^7 and 4.89×10^7 respectively. However, total oocyst output for the

oocysts stored in CHX digluconate was significantly ($P<0.05$) lower than the oocysts stored in 2.5% PDC and CHX gluconate at 2.95×10^7 . Total oocyst output post-challenge with 100% AF oocysts was 0 for all groups excluding the 2.5% PDC (2.22×10^4). These findings suggest that CHX gluconate could be a potential alternative to PDC for *in vitro* storage of *Eimeria* spp. oocysts and that AF% can be used as a reliable tool to assess EMM6 viability after artificial aging.

Key Words: coccidiosis; *Eimeria maxima*; chlorhexidine; autofluorescent; potassium dichromate

11 Characterizing the impact of *Cochlosoma anatis* infection on gut health and performance in turkeys. Justin Lowery^{1*}, Catherine Fudge¹, Katherine Cupo¹, Christina Sigmon¹, Lin Walker¹, Chongxiao Chen¹, ¹North Carolina State University, Raleigh, North Carolina, United States.

Cochlosoma anatis is a flagellated protozoan parasite impacting the turkey industry. Infection with *C. anatis* is associated with runting, diarrhea, and depression, with high morbidity in infected flocks. While these symptoms do not typically lead to bird death, farmers may experience high monetary losses. This study was multi-faceted and focused on the effects of *C. anatis* infection on growth performance, intestinal permeability, and blood chemistry in turkeys. One-day-old turkey poults were grown in 16 isolation cages for 2 weeks. Then, the birds were redistributed into 16 isolation cages with 10 birds per cage to achieve a similar total weight between cages. Eight cages were challenged with $\sim 5 \times 10^5$ *C. anatis* cells/bird (PC), while the remaining cages were not infected (NC). Body weight (BW) and feed intake (FI) were recorded at weeks 2 and 4. Feed conversion ratio (FCR) was calculated from weeks 2 to 4. Individual body weight was recorded at the end of the trial to assess cage uniformity. At week 4, intestinal permeability was tested using FITC-d orally administered and its blood concentration was measured. Blood chemistry analysis was also performed using an iStat1 blood analyzer at week 4. The data generated from these tests were analyzed using a t-test in SAS 9.4. Significant difference was determined when $P<0.05$. The poults used in this study exhibited all symptoms associated with *C. anatis* infection. The results showed that poults infected with *C. anatis* did not experience significant changes in intestinal permeability ($P=0.21$) or flock uniformity ($P=0.13$). However, FI ($P<0.01$) and BWG ($P<0.01$) were both decreased significantly in the PC group. Although not statistically different, the FCR of the infected group trended lower than the control ($P=0.08$). Moreover, infected poult blood chemistry revealed significant increases in extracellular fluid ($P<0.01$), bicarbonate ($P<0.01$) and carbon dioxide levels ($P<0.01$) compared to the NC group. In conclusion, these results indicate that *C. anatis* challenge decreases BWG and FI of poults, reflecting historical observations, but no changes were observed in uniformity and intestinal

permeability. Evidence of elevated extracellular fluid and bicarbonate in the blood indicate dehydration, likely due to diarrhea causing the loss of acid and water from the body and the need to regain homeostasis. Further research should focus on the impact of *C. anatis* infection on nutrient utilization and immune response. Choosing this objective should contribute to understanding performance reduction during *C. anatis* challenge and help to develop a strategy to alleviate performance loss during production.

Key Words: Performance; Blood Chemistry; Permeability; *Cochlosoma anatis*; Uniformity

12 A method to evaluate anticoccidial drug resistance. Jonathan Pierron^{2*}, Carl Julien¹, ¹CRSAD, Deschambault, Quebec, Canada, ²Université Laval, Québec, Quebec, Canada.

The aim of this study was to develop a method to evaluate *Eimeria* resistance to anticoccidials. Six anticoccidial drugs (amprolium, decoquinate, monensin, nicarbazin, salinomycin, zoalene) were evaluated in triplicate on the *Eimeria* pathogenic cycle by investigating oocyst sporulation and sporozoite viability and host cell invasion. Two 35-day floor pen trials with 9 replicates of 55 birds/pen were also conducted in broilers using the same drugs. Non-infected (NI) and *Eimeria*-infected (EI) birds were used in both trials. *Eimeria* infection was induced by an oral administration at d14 of 200,000 sporulated oocysts of a population of mixed *Eimeria* species harvested from field isolates. Performance data and feces oocyst excretion counts were analyzed by a mixed model including treatment as fixed effect and barn section as random effect. Intestinal lesion scores were analyzed by Kruskal-Wallis tests using two birds per pen ($n=18$ per treatment). For *in vitro* experiments, an *Eimeria* population from Coccivac®-B52 (Merck Animal Health) was also used as a drug-sensitive control. Using the field *Eimeria* population, *in vitro* tests showed a dose-response effect of anticoccidials (0.5, 12.5, 50 and 100 $\mu\text{g/mL}$) on decreasing sporozoite viability, reaching -60% ($P<0.0001$), oocyst sporulation (-80% , $P<0.0001$) and host cell invasion (-90% , $P<0.0001$). However, on host cell invasion, amprolium presented non-linear variations depending on the exposure concentrations (from -37% to -92%) and zoalene showed a trend to reduce efficiency with higher concentrations (from -95% to -67%). In both broiler trials, EI reduced body weight (BW) 6 days post-infection (6 dpi, d20), worsened feed conversion ratio (FCR) during the grower period (10-20d), *Eimeria* excretion at 6 dpi and 7 dpi intestinal *E. tenella* lesion score, compared to NI birds ($P<0.01$). Decoquinate and monensin prevented the *Eimeria* challenge-induced BW loss and FCR, OPG and intestinal lesions increases. Whereas blocking OPG and intestinal lesions increases, salinomycin and nicarbazin showed adverse effects on BW with similar numbers at d20 ($P>0.05$) and with decreases at d34 (2.45-2.48 vs 2.61 kg, $P=0.0009$), compared to EI. Zoalene and amprolium

showed total OPG increases (8.7 and 7.5 vs 1.6 10^5 OPG, $P<0.003$), compared to NI, and did not prevent intestinal *E. acervulina* lesions (1.7 and 1.2 vs 1.3, $P>0.05$), compared to EI. qPCR analyses also confirmed that zoalene and amprolium did not reduce excreted *E. acervulina* ($P>0.05$), compared to EI. Overall, the *E. acervulina* strain in the tested field *Eimeria* population

seemed to be resistant to amprolium and zoalene. This *in vitro* method could be used to evaluate the sensitivity of anticoccidials and their alternatives.

Key Words: Broilers; Coccidiosis; Intestinal health; Models; Drug resistance

Student Competition: Genetics and Molecular Biology I

13 Identification of *cis*-acting elements essential for glucocorticoid induction of growth hormone gene expression in chicken embryonic pituitary cells. Kuan Ling Liu^{1*}, Tom Porter¹, ¹*University of Maryland, College Park, Maryland, United States.*

Glucocorticoid (GC) treatment of rat or chicken embryonic pituitary (CEP) cells induces premature production of growth hormone (GH). The GH gene lacks a canonical GC response element (GRE), and its induction by GC requires ongoing protein synthesis. Some *cis*-acting elements and *trans*-acting factors have been identified. One GC-responsive region (GCRR) is located at -1045/-964 of the chicken GH gene, which contains an ETS-1 binding site and a degenerate GRE half site. Binding of ETS-1 and GC receptor (GR) to these elements are required for corticosterone (CORT) induction of the GH gene in e11 CEP cells. Downstream of the GCRR, deleting the intermediate sequences between -953 and -650 of the GH gene reduces CORT responsiveness. Our objective was to identify additional *cis*-acting elements essential for GH promoter activity in response to CORT. We cloned a fragment of the chicken GH gene (-1724/+25) into a luciferase reporter and transfected into e11 CEP cells. We measured the promoter activity in fold changes of CORT over basal treatment. Statistical analysis was conducted using a one-way ANOVA, and Tukey's HSD was performed to determine significant differences among groups. We narrowed the region of interest (-953/-650) to -952/-890 through promoter deletions ($P \leq 0.001$; $n=4$). This region includes a putative distal (d) transcription factor ETS-Like 1 protein (ELK-1) binding site (-947/-934), and the mutation of this dELK-1 binding site in the -1742/+25 GH reporter had a 70% reduction in activity compared to the full length GH reporter ($P \leq 0.001$; $n=4$). We continued to identify four additional *cis*-acting elements from -650 to +25 of the GH gene. Mutation of the medial (m)ETS-1 (-464/-455), proximal (p)ELK-1 (-360/-347), pPIT-1 (-136/-127), and pETS-1 (-22/-13) in the -1724/+25 GH reporter all had lowered CORT responsiveness ($P \leq 0.001$; $n=4$). These results indicate that multiple response elements for members of the ETS family of transcription factors are required for CORT induction of GH transcription. We aim to characterize these essential regulatory elements by identifying the *trans*-acting factors that bind to these regions. Revealing CORT-inducible *trans*-acting factors for the GH gene will contribute to better understanding of endogenous GH regulation as well as its downstream targets in chickens.

Key Words: corticosterone; development; somatotropin; ETS-family of transcription factors; PIT-1

14 Identification of tissue-specific transcription factor binding motifs in chicken strong enhancers.

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Chicken meat is currently the most consumed animal protein in the world. Genetic improvement in economically important traits has enabled the industry to meet increasing consumer demand. Identifying causal variants underlying these large genetic gains will improve genomic selection for these agronomic traits. The regulatory elements including strong enhancers play a significant role in modulating the gene expression. Genetic variants within the transcription factor binding motifs (TFBMs) of strong enhancers are the major contributing mechanism for higher production. Hence, the objective of this study was to identify TFBMs that were enriched in strong enhancers of respective tissues. Previously, our lab garnered an atlas of regulatory elements in the chicken genome from 23 tissues and categorized them into 26 tissue-specific strong enhancers including each individual tissue, all common (across all tissues), brain common (across three brain tissues), and intestine common (across four intestine tissues). Here, HOMER (v4.11) was used to identify tissue-specific TFBMs that were significantly enriched in strong enhancers with a cutoff false discovery rate of less than 0.05. The results suggest that there are many notable tissue-specific TFBMs such as NF1& SOX6 in the brain, MEF2C in the muscle, and HNF1B & HNF4G in the liver. These findings were consistent with previously published results in human and pig genomes. Remarkably, IRF8, an important transcription factor for B cell development, has its motif specifically enriched in the bursa – a lymphoid organ in the chicken. Further footprinting analysis using the ATAC-seq signals will be conducted to confirm the TFBMs. In conclusion, the identified TFBMs in tissue-specific strong enhancers are highly relevant to the corresponding tissue function. These findings not only help us predict tissue-specific transcription factors to study gene regulatory networks but also serve as an important tool for researchers to design targeted gene expression experiments to decipher the link of genotype to phenotype in chickens.

Key Words: Chicken; Transcription factor binding motif; Tissue-specific regulatory elements; Enhancer

15 Functional Validation of Candidate Enhancers in the Chicken Genome using Massively Parallel Reporter Assays. Liqi An^{1*}, Ying Wang¹, Zhangyuan Pan¹, Dailu Guan¹, Hans Cheng², Huaijun Zhou¹, ¹*UC Davis, Davis, California, United States*, ²*USDA-ARS, East Lansing, Michigan, United States.*

With the generation of the chicken genome, efforts have switched to genome annotation, especially the identification of tissue-specific enhancers given their important role in regulating gene expression. As part of the FAANG effort, our lab has identified thousands of candidate enhancers in

the chicken genome through *in silico* prediction. While an important first step, the FAANG annotated enhancers may have low concordance with their *bona fide* functional activities, thus, experimental validation of enhancer activity is needed. In this study, we aim to develop high-throughput functional validation of enhancers in the chicken genome. We first validated two candidate splenic-specific enhancers, whose target genes encode Prolyl 4-Hydroxylase Subunit Beta and Migration and Invasion Inhibitory Protein, using the standard luciferase reporter assay in the DF-1 chicken cell line. We are currently developing massively parallel reporter assays (MPRAs) in DF-1 cells to be able to scale up our screens. Our candidate enhancers include two sets of predicted strong enhancers: (1) whose target genes show high expression in DF-1 cells, and (2) whose target genes show allele-specific expression related to genetic resistance to Marek's disease. Our MPRA library involves the synthesis of ~2,000 putative enhancers each coupled to unique barcodes. Positive and negative control sets are also included. The development of this high throughput enhancer validation system will enable the genome-wide discovery and functional characterization of enhancers in the chicken genome, which should provide a critical knowledge base for the systematic exploration of their roles in chicken biology and disease resistance.

Key Words: Enhancer; chicken genome; MPRAs; reporter assay; high-throughput

16 Characterizing the alteration in insulin-responsive genes in chicken pectoralis through transcriptome analysis. Xiangli Zhang^{1*}, Pengfei Du¹, Ziyang Wang¹, Lin.Ge Gao¹, Huaiyong Zhang¹, W Chen¹, Y.Q. Huang¹, ¹*College of Animal Science and Technology, Henan Agricultural University, Zhengzhou, Henan, China.*

Although emerging evidence indicated that insulin-responsive genes are of considerable significance in both development and metabolism among mammals, whereas the potential function of insulin-responsive genes in the development and metabolism of skeletal muscle remains poorly understood in chicken. In the present study, the mRNAs related insulin-responsive were identified in chicken pectoralis using transcriptome analysis. With this aim, forty 24-day-old male Arbor Acres broilers were randomly divided into 2 groups and received intraperitoneal injection of 5 IU/kg insulin or phosphate buffered saline (PBS) after 16 h of fasting. Pectoralis was collected for RNA sequencing at 15 min and 120 min after injection. Regarding the analysis and identification of differentially expressed genes (DEGs) by DESeq (*padj* < 0.05; |Fold Change| ≥ 2), showed that compared with the PBS group, only 2 DEGs response to insulin after injection 15 min, 592 DEGs were screened after insulin injection 120 min, furthermore, compared to insulin injection 15 min, 594 DEGs were screened after insulin injection 120 min; thereinto, 410 DEGs were sensitive to both insulin administration and manipulated time; regarding to 778

insulin-responsive DEGs (i.e., 553 up-regulated and 225 down-regulated after insulin administration), the outcomes from GO and KEGG enrichment analysis indicated that these insulin-responsive DEGs mainly involved in metabolism and muscle development. Besides, 11 insulin-responsive DEGs were randomly selected for expression verification by qPCR. The relative expression levels of RNAs were normalized with β -actin and calculated using the comparative $2^{-\Delta\Delta CT}$ method, and two-tailed unpaired *t*-test were used. The results were consistent with the fluctuation in the sequencing data. Specifically, it was found that insulin inhibited the expression of insulin like growth factor (including *IGF1* and *IGF2*) and promoted the transcription of insulin receptor (*INSR*), phosphoinositide-3-kinase regulatory subunit 1 (*PIK3RI*), forkhead box O3 (*FOXO3*), solute carrier family 7 member 5 (*SLC7A5*), 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (*PFKFB3*), pyruvate dehydrogenase kinase 4 (*PDK4*), and pyruvate dehydrogenase phosphatase catalytic subunit 1 (*PDP1*). It is well-known that *PFKFB3* and *PDP1* are associated with glycolysis and glucose oxidizing, as well as *SLC7A5* involved in neutral amino acid transport. Therefore, the current study shows that insulin produces a wide range of effects on the development and metabolism of chicken pectoralis, which combines with *INSR* to upregulate glycolysis and the transportation of amino acids in pectoralis of chicken through activating PI3K and/or FOXO signaling pathway.

Key Words: chicken; insulin; transcriptomics; pectoralis

17 Study on the expression characteristics of pyruvate dehydrogenase kinase 4 in broilers and its response to exogenous insulin and restricted feeding. Jingya Jiao^{1*}, Lin.Ge Gao¹, Huihong Li¹, H.Y Zhang¹, W Chen¹, Y.Q. Huang¹, ¹*Institute of Animal Science and Technology, Henan Agricultural University, Zhengzhou, China.*

Pyruvate dehydrogenase kinase 4 (*PDK4*) has been shown to be the center of pyruvate dehydrogenase complex (PDC) regulating substance metabolism and play key role in pyruvate metabolism by inhibiting PDC activity to provide energy source for cell growth, implying that the regulation of *PDK4* could directly affect the efficiency of glycolysis. Therefore, this experiment was conducted to detect the *PDK4* expression in different tissues responses to exogenous insulin and feed restriction in broilers. The 24-d-old broilers were intraperitoneally injected with insulin (5 IU/kg) or phosphate buffer saline (PBS). Tissue samples were collected at different time points (0, 15, 120, and 240 min, n = 5) after injection, and the expression of *PDK4* in different tissues was detected at 0 min and corresponding time points after injection. In addition, to evaluate the response of the expression of *PDK4* to feed restriction, 7 day-old broilers were randomly divided into 3 groups: control group free to access to conventional diet, energy restriction group (15% energy restriction), or protein

restriction group (15% protein restriction) the experiment lasted until 21 days of age. Similarly, twenty 16-d-old broilers were randomly selected for fasting for 24 h and freely feeding experiment. The pectoral muscle was gained for the determination of the expression of *PDK4*. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons. Results showed that the *PDK4* expression was the lowest in chest muscle, liver and leg muscle at E14 stage ($P < 0.05$), and it upregulated gradually with the increase of embryonic age in the pectoralis muscle. In the liver, the *PDK4* expression was highest at the end of embryonic development ($P < 0.05$), and significantly downregulated after hatching ($P < 0.05$). However, the *PDK4* expression was significantly upregulated in leg muscle at D7 and D21 stages ($P < 0.05$). Response to insulin administration, the *PDK4* expression level was significantly increased at 120 min in pectoral muscle by insulin injection as compared with PBS group ($P < 0.05$), whereas the *PDK4* expression level was significantly decreased in abdominal fat at 240 after insulin injection ($P < 0.05$). In this study, neither 15% energy restriction nor 15% protein restriction did change the expression of *PDK4* in pectoral muscle ($P > 0.05$). However, 24 h fasting significantly increased the expression of *PDK4* in the breast muscle of broilers ($P < 0.05$). In conclusion, these studies indicate that *PDK4* expression varied in different developmental stages and tissues, and both insulin injection and 24 h fasting increased *PDK4* expression in breast muscle of broilers.

Key Words: chicken; *PDK4*; restriction; insulin

18 Molecular cloning and expression characterization of chicken Glucose transporter protein 4 (*GLUT4*). Pengna Luo^{1*}, Chuanchen Su¹, Ziyang Wang¹, Huihong Li¹, H.Y. Zhang¹, W. Chen¹, Y.Q. Huang¹, ¹*Henan Agricultural University, ZhengZhou, Henan, China.*

Glucose transporter protein 4 (*GLUT4*) plays an important role in regulating insulin-mediated dynamic balance of glucose metabolism in mammals. Until now, studies on *GLUT4* have focused on mammals mostly, while chicken *GLUT4* has been rarely investigated. In this study, we cloned the sequence of chicken *GLUT4* and analyzed its expression characterization. The *GLUT4* sequence of chicken was obtained by combining conventional amplification, 3'-rapid amplification of cDNA ends (RACE) and 5'-RACE, and then bioinformatics analysis was carried out. In addition, the distribution of *GLUT4* in 24-day-old Arbor Acres broilers and the changes of *GLUT4* expression level in insulin-sensitive tissues after abdominal subcutaneous injection of 5 IU/kg insulin were successfully collected and four samples per group were used for qPCR. One-way ANOVA was used to analyze data with multiple groups, Students' *t* test was used to analyze the difference between two groups. Results showed that chicken *GLUT4* gene has at least 14 transcripts predicted encoding 12 types of amino

acid sequences (ranged from 65 AA to 519 AA). All transcripts can be mapped to the chicken genome. The predicted proteins had typical MFS domain of glucose transporters. However, subcellular localization of different proteins varied widely. The predominant transcript (named as T1), consisted of 11 exons with an open reading frame (ORF) of 1560 bp encoding 519 amino acids. Analyzing the expression distribution of *GLUT4* in the tissues, it was found that *GLUT4* was constitutively expressed in the pectoralis, leg muscle, heart, liver, glandular stomach, brain, lung, kidney and abdominal fat, with the highest mRNA level in pectoralis. Furthermore, after insulin injection, the expression of *GLUT4* showed an upward trend in the pectoralis, leg muscle, liver and abdominal fat with time. *GLUT4* expression at 240 min was significantly higher than 0 min in pectoralis and abdominal fat ($P < 0.05$). Compared with PBS group, after 240 min of insulin treatment, the expression level of *GLUT4* in leg muscle was significantly upregulated ($P < 0.05$). These data indicated that chicken *GLUT4* gene might undergo complex transcription regulation and may be involved in glucose metabolism after insulin treatment. The study provided a basis for further understanding of glucose homeostasis in birds which is still enigmatic.

Key Words: insulin; *GLUT4*; gene cloning; bioinformatics analysis; expression

19 Expression characteristics of Chicken Sestrin 1 and its response to exogenous stimulus. Huihong Li^{1*}, Binghao Shao¹, Jingya Jiao¹, Huaiyong Zhang¹, Y.Q. Huang¹, ¹*Henan Agricultural University, Zhengzhou, China.*

Sestrin (*SESN*), an insulin- and glucose-sensitive gene, involves in the regulation of aging, muscle development, nerve degeneration, and carcinogenesis. However, the expression of *SESN1* gene is rarely studied in chickens. The purpose of this study was to identify the spatio-temporal expression of *SESN1* in chicken and its response to exogenous glucose, insulin, sodium pyruvate, and restricted feeding. Data were analyzed using One-way analysis of variance (ANOVA). The outcomes from the spatio-temporal expression characteristics of *SESN1* at different growth stages (E10, E19, 21 d, 49 d, $n = 4$) showed that the relative expression levels of *SESN1* were the highest in the pectoralis muscle of 21 d chicken, followed by leg muscle and pancreas ($P < 0.01$). The expression level of *SESN1* dramatically varied in the pectoral muscle of birds at different growth stages, with the highest level at E19 and the lowest at E10 ($P < 0.01$). Subsequently, the expression of *SESN1* were explored with the pectoral muscle of broilers collected at different time points after intraperitoneal injection of glucose (18 d, $n = 6$), sodium pyruvate (21 d, $n = 6$) or insulin (24 d, $n = 6$). Here, up to 120 min after insulin treatment, the expression of *SESN1* increased obviously and then began to decrease. In addition, the transcription of *SESN1* was significantly higher at 120 min than the basal state or phosphate buffer

saline group ($P < 0.01$). After sodium pyruvate injection, the expression of *SESNI* in the pectoral muscle showed a significant increase in 1 h. Nevertheless, under the stimulation of exogenous glucose, the expression of *SESNI* did not change significantly in the first 10 min, but it significantly decrease at 60 min. Response to restricted feeding, 15% protein restriction resulted in the decreased expression of *SESNI* in breast muscle when compared with the control group ($P < 0.05$). In summary, these results suggested that the relative expression levels of *SESNI* was higher in the pectoralis muscle of chicken, which might be involved in the gluconeogenesis of breast muscle.

Key Words: breast muscle; chicken; *SESNI*; exogenous intervention

20 Effect for introduction Rhode Island Red genes on the related indexes of Henan fighting chickens. Chuanchen Su^{1*} Pengna Luo¹ Yongshuai Wang¹ H.Y Zhang¹ Wen Chen¹ Y.Q. Huang¹, ¹*Henan Agricultural University, Zhengzhou, Henan, China.*

Henan fighting chickens is a famous Chinese cockfighting breed, which had strong aggressive. This experiment aims to investigate the effect for the introduction of 25% genes from Rhode Island Red chickens on the related indexes of Henan fighting chickens through the comparisons between 120-day-old pure Henan fighting chickens (PC, n=10) and its hybrid chickens (introduced into 25% Rhode Island Red genes, HC, n=10), half male and female were used for the related research in each breed respectively. The serum biochemical parameters were measured, and the muscle fiber features were detected from the pectoralis and leg

muscles of birds. All data were analyzed by SPSS23 for One-way ANOVA and double-factor variance analysis. Results showed that for the serum biochemical indexes, the globulins (GLO) ($P < 0.01$), cholesterol (CHO) ($P < 0.01$) and LDL-C ($P < 0.05$) in the HC roosters were significantly higher than those in the PC roosters, and there was significantly gender effect for triglycerides (TG) (rooster is higher than the hen, $P = 0.002$). In pectoralis, muscle fiber diameter had significant population effects (HC is higher than PC, $P = 0.014$) and gender effects (rooster is higher than the hen, $P = 0.013$); The muscle fiber density in the HC roosters was significantly higher than that in the PC roosters ($P < 0.01$). In leg muscles, the muscle fiber density presented significant interaction effect between population and gender, it was significantly higher in the PC roosters than that the HC roosters and the female PC chickens ($P < 0.01$); While it was significant lower in the female PC birds than that the female HC birds ($P < 0.05$). The MyHC (Myosin Heavy Chain) and MyoG (myogenin) genes are genes associated with fiber development. The qRT-PCR results showed that the MyHC mRNA level in the HC roosters was higher than that in the PC roosters ($P = 0.057$) in pectoralis. MyHC mRNA levels in female HC chickens were significantly higher than that female PC chickens ($P < 0.01$) in leg muscle. In addition, there were no significant differences between two breeds for the expression of MyoG gene in both pectoralis and leg muscles of birds. It shows that the introduction of 25% Rhode Island Red genes changed the serum indexes and muscle fiber traits of Henan fighting chickens in sex-specific manner.

Key Words: Rhode Island Red; qRT-PCR; Henan fighting chickens; serum biochemical indicators; fiber characteristics

Student Competition: Animal Well-Being and Behavior

21 Tracking Pecking Behaviors and Damages with Machine Vision in Cage-free Hen Houses. Sachin Subedi^{1*}, Lilong Chai¹, Ramesh Bist¹, Xiao Yang¹, ¹*University of Georgia, Athens, Georgia, United States.*

Feather pecking is one of primary welfare issues in cage-free hen houses. Pecking can seriously reduce the well-being of birds and causes economic losses for the egg producers. After beak trimming is a highly criticized in Europe and the USA, alternative methods are needed for pecking monitoring and management. A possibility for minimizing the problem is early detection of feather pecking behaviors and damages to prevent it from spreading or increasing. Real-time monitoring of feather pecking and warning is a possible solution. The objectives of this study were developing a machine vision method and testing the performance of the model in tracking pecking behaviors of chickens and potential damages on injured birds. Object detection is a significant branch of computer vision and image processing. In recent years, there have been advancements in deep learning-based object detection methods. YOLO (You Only Look Once) is an advanced object detection technology with very high precision and speed compared to CNN-based algorithms (e.g., R-CNN, Faster R-CNN, and Mask R-CNN, etc.). In this study, we proposed a “YOLOv5 network to detect feather pecking of laying hens in research cage-free facilities. A dataset of 1924 images (1300 training, 324 validations, and 300 test) from 22-28 weeks of age was created to analyze the feather pecking behavior. We manually annotated the images using “Makesense.ai” an open-source image annotation tool, and we saved the annotated files in txt format. A rectangular shape bounding box was drawn to the object indicating the feature of “Pecking.” For data analysis, we divided the data files into two folders: images and labels, with each folder having two subfolders: train and valid. Pre-trained YOLOv5 algorithm trained our dataset. We took 300 unlabeled images as a test dataset for detection. Results show that the trained algorithm can detect the feather pecking behavior with high confidence. We trained our datasets with batch size of 32 for 180 epochs using Google Colaboratory. The precision, recall and mean average precision (mAP) of the model for training and detection were 0.701, 0.407, and 0.485, respectively. Getting the best image quality in farm conditions is somewhat tricky as there is an accumulation of dust on the farm and the crowding of birds. This affects our model to get the best information from our data and overall accuracy. This current study is novel, and there have been no previous studies that used computer vision methods to detect feather pecking behavior. We are currently improving the model to detect the pecking more accurately.

Key Words: animal welfare; feather pecking; precision farming; Laying hen production; deep learning

22 A deep learning model for detecting hens on litter floor of cage-free houses. Xiao Yang^{1*}, Lilong Chai¹, Ramesh Bist¹, Sachin Subedi¹, ¹*University of Georgia, Athens, Georgia, United States.*

Real-time and accurate detection of chicken is the cornerstone of precision farming based on image recognition. However, such identification becomes more challenging under cage-free situation comparing to caged hens. In this study, we developed a deep learning model based on YOLOv5 and YOLOX, two advanced convolutional neural networks (CNN) for monitoring hens' behaviors under cage-free facilities. More than 1000 images were used to train the model and additional 200 images were adopted to test it. The one-way ANOVA and Tukey HSD analysis were conducted using JMP software (JMP Pro 16 for Mac, SAS Institute, Cary, North Carolina) to determine whether there are significant differences between predicted number of hens and actual number of hens under various situations (i.e., age, light intensity, and observational angles). The difference was considered significant at $p < 0.05$. Our results show that the evaluation metrics (Precision, Recall, F1 and mAP@0.5) of the model are 0.96, 0.96, 0.96 and 0.95, separately, in detecting hens on floor. The model has stable performance in detecting birds under different lighting intensities, angles, and ages over 8 weeks. For instance, the model was tested with 95% accuracy after birds were 8 weeks old. However, younger chicks such as one-week old birds were hard to be tracked with only 25% accuracy due to interferences of equipment such as feeders, drink lines, and perches. According to further data analysis, the model performed efficient in real-time detection, which is the key step for individual birds' tracking for evaluation of production and welfare. However, there are some limitations for the model. Error detections came from highly overlapped stock, uneven light intensity, and images occluded by equipment (i.e., drinking line and feeder). Future research is needed to address those issues for a higher detection. Current study establishes the first real-time and accurate CNN model under cage-free facilities for birds' detection, which provides technical basis for developing a machine vision system for tracking individual birds for evaluation of behavior and welfare status.

Key Words: Egg production; precision farming; cage-free system; deep learning algorithms

23 Effects on Production, Welfare, and Behavior on Layers Transferred from a Caged to Cage-free Environment. Gerald Self^{1*}, Ishab Poudel¹, Pratima Adhikari¹, ¹*Mississippi State University, Starkville, Mississippi, United States.*

A six-week study was conducted to evaluate the behavior, welfare, and production effects on laying hens with a history of cage system after being transitioned to a cage-free

environment. A total of 84 Hy-Line W-36 laying hens at the age of 70-weeks were used in the experiment. Each pen consisted of seven birds and contained identical littered floors, waterlines with nipple-drinkers, hanging feeders, and wooden perches. Furthermore, each pen also contained a Hikvision® Night Vision camera to observe the behaviors of the hens at various points of time during the day. Hens were adapted for one week for acclimation to the cage-free environment. The production performance data calculated were the following: feed intake (FC), hen-day egg production (HDEP), hen-day egg mass (HDEM), and feed conversion ratio (FCR-gram of feed consumed per gram of egg). The production performance data was analyzed as in a completely randomized design (CRD) comparing with weeks as a factor, with PROC GLM procedure SAS 9.4. Furthermore, four welfare evaluations were also conducted throughout the trial evaluating the paw, comb, and feather scores. There were no differences in HDEP, HDEM, and average egg weight. Welfare data was analyzed with PROC FREQ procedure and subjected to Fisher's Exact test. The positive welfare results opposed the hypothesized negative results as the trial was conducted. Paw scores remained consistent throughout the trial with a lesion percentage of 4.86% to 6.08% through the four scoring evaluations. Feather lesion percentage initially began at 6.99% but decreased to 4.26% by the 73rd week; however, after this the lesion percentage rose to 11.55% (P=0.0005). Comb lesion percentage remained at 5.78% through the first two scoring evaluations, but steadily decreased to 3.95% by the final evaluation (P=0.0733). The two main behaviors observed from the transferred hens were preening and foraging. Preening accounted for 45% of the hens' daily activity, and foraging accounted for 44%. Furthermore, the hens' activity also exhibited minor behaviors of perching, dustbathing, and brooding. Thus, contrary to the hypothesis, hens require longer than one month for adaptation to their new environment. It may take up to four additional weeks for hens to adjust and acclimate to their new environment. Furthermore, the stress caused from the environmental and pen mate changes may have induced molting within the 70-week-old hens. As consumer preference towards alternatively produced eggs in the market becomes more prevalent, further extensive studies that evaluate welfare become more essential to continue the growth of the egg industry.

Key Words: Laying Hen; Behavior; Cage-free

24 Strain, age, and maternal fed omega-3 fatty acids influence egg-laying chickens in a discrimination task. Rosemary Whittle^{1*}, Alexandra Harlander¹, David Ma¹, Elijah Kiarie¹, Tina Widowski¹, ¹University of Guelph, Guelph, Ontario, Canada.

Maternal fed omega-3 fatty acids (n-3 FA) increase cognition in offspring of many species, including mice, partridges, and primates. Supplementing hens' diets with flaxseed n-3 FA high in alpha-linolenic acid, may benefit

embryonic brain development. This study aimed to determine if a maternal flaxseed diet would alter the performance of offspring in a discrimination task. Parent flocks (Shaver white and ISA brown) were fed a control (C, n6: n3 ratio = 14.7) or 2.6% co-extruded full-fat flaxseed diet (Flax, n6: n3 ratio = 5.3). Offspring were housed in 48 enriched floor pens (12 pens per strain/treatment combination). At 12 (pullets, pre-laying) and 33 (hens, egg-production) weeks old, naïve birds were selected for a discrimination task with 3 stages: training (N=437), discrimination test (DT) (N=350), and reversal learning (RL) (N=213). Training had five phases (P): P1 – eat from clear cup, P2 – eat from coloured cup, P3, P4, and P5 – remove lid and eat from half, 3-quarter, and fully covered cup respectively. Pass criteria for training - 4/5 completed tasks in each phase. In DT and RL, birds were presented two cups simultaneously, with food in one coloured cup (green or blue). The pass criteria were 4/5 correct choices for DT and RL for 3 and 2 consecutive days. In DT and RL, birds not reaching criteria in 9 days were excluded. Data were analysed using survival analyses, determining the hazard ratio (HR), upper (CIH) and lower (CIL) confidence intervals, and p-values. There was a significant effect of age for P1 (HR=1.55, CIL=1.15, CIH=2.09, p<0.001), P2 (HR=1.86, CIL=1.21, CIH=2.86, p=0.004), P3 (HR=1.54, CIL=1.00, CIH=2.38, p=0.049), DT (HR=1.33, CIL=1.01, CIH=1.74, p=0.042) with hens more successful than pullets and tending to be more successful in P4 (HR=1.91, CIL=0.99, CIH=2.62, p=0.054). In RL pullets were more successful than hens (HR=0.44, CIL=0.23, CIH=0.86, p=0.016). There was a significant effect of strain in P4 (HR=1.74, CIL=1.02, CIH=2.98, p=0.043) and DT (HR=1.33, CIL=1.01, CIH=1.75, P=0.042), white chickens were more successful. There was an interaction between treatment and strain in P3, brown/Flax chickens were most successful (HR=2.01, CIL=0.96, CIH=4.18, p=0.036). There was a significant interaction between strain and age for P1 (HR=2.28, CIL=1.48, CIH=3.52, p<0.001) and P3 (HR=2.09, CIL=1.09, CIH=3.99, p=0.026), brown/hens were most successful. These results show that age, strain, and their interaction affected the performance of layers in this learning task. Supplementing maternal diet with n-3 FA had little effect on the learning rates or success in this task.

Key Words: Behaviour; Laying hen; Maternal diet; Omega-3 fatty acids; Cognition

25 Heterophil to lymphocyte (H:L) ratios are an inaccurate short term measure of acute stressors in laying hen pullets. Meagan Abraham^{2*}, Gregory Fraley², Priscila Serpa¹, Darrin Karcher², ¹Virginia Tech, Blacksburg, Virginia, United States, ²Purdue University, West Lafayette, Indiana, United States.

The pullet phase accounts for 25% of a laying hen's life and management choices during this phase can potentially affect a hen's lifetime productivity and welfare. However, there has been limited research on management, welfare, and the

stress response of pullets. As the laying hen industry develops new housing styles and attempts to identify best practices for management and welfare in extensive housing, there is an immediate need to accurately evaluate stress during the pullet phase. Historically, heterophil to lymphocyte (H:L) ratios have been used as a proxy to measure stress levels in poultry. Elevated H:L ratios result from decreased proportions of lymphocytes in circulation in response to corticosteroids. This may signal reduced welfare, but it is unclear if these same H:L elevations occur during the pullet phase. A factorial crossover design was used to artificially activate the hypothalamic-pituitary-adrenal (HPA) stress pathway to understand changes in the stress response during a hen's lifetime. Ten birds were injected with 0.125 mg/kg Cosyntropin™ (ACTH) or a control (mannitol-saline) solution at 6, 17, and 26 weeks of age (WOA). Blood was collected into heparinized syringes before the treatment injection and one and two hours post-injection. A log-transformed, linear model of treatment, age, time after injection and interaction effects was evaluated. Age, time after injection, and age*treatment effects were found ($P < 0.01$). Comparing ACTH versus control treatments at each age found no difference ($P > 0.05$) within an age group although an age*treatment interaction was found ($P < 0.01$). The highest average H:L ratios, 0.321, were observed at 26 WOA and the lowest were recorded at 17 WOA, 0.006. Average H:L ratios at 6 WOA were 0.016. Based on Tukey's method of multiple comparisons, differences between ages were significant ($P < 0.05$). H:L ratios increased, regardless of treatment, as time from the initial injection increased. Mean H:L ratios were 0.020, 0.027, and 0.054 at 0, 1, and 2 hours post-injection, respectively. Pairwise comparisons using Tukey's method found differences when comparing 0 and 2 hours post-injection only ($P = 0.03$). Chronic handling stress was likely causing the elevations in H:L ratios at 2 hours post-injection and the dip in H:L ratios at 17 WOA is likely due to hormonal and physiological changes as the pullet reaches sexual maturity. This illustrates caveats to evaluation of stress during the critically important pullet phase. H:L ratios were not affected by short-term HPA axis activation, using ACTH, within 2 hours of a stressor. H:L ratios were not an accurate measure of acute stress in pullets.

Key Words: Stress; Welfare; Heterophil; Lymphocyte; Pullet

26 Characterizing age-related changes in walking ability and welfare of individual broiler chickens with sound and poor gait and the effects of stocking density in commercial barns. Hailee Yoder^{1*}, Anja Riber², Tomas Norton³, Marisa Erasmus¹, ¹Purdue University, West Lafayette, Indiana, United States, ²Aarhus University, Aarhus, Denmark, ³Katholieke Universiteit Leuven, Leuven, Belgium.

Lameness is a costly welfare concern of broilers which can lead to additional welfare concerns and lost productivity.

While it is known that walking ability declines with age and increasing stocking density, little is known about whether individual birds initially classified as having sound or poor gait experience similar declines in walking ability. This study examined 1) how walking ability (gait) and welfare measures of individual broiler chickens that were originally classified as having poor or sound gait changed with age, and 2) the influence of stocking density on changes in gait and welfare measures. Four pens (4ft x 10 ft), representing 4 groups were constructed in each of 4 commercial barns (2 barns/farm; $n = 4$ pens/group). Broilers (Ross 708, 196 birds/barn) were randomly assigned to pens based on their gait at 33d. The four groups were: S, birds with sound gait housed at farm stocking density (6lb/ft², 56 birds); P, birds with poor gait at farm density; MF, mixed (sound and poor) gait at farm density; and ML, mixed gait at low stocking density (3lb/ft², 28 birds). Sound gait was classified as a score of 0-1 on the Bristol Scale (Kestin et al., 1992), while poor gait was classified as a score of 2+. Completely lame birds were excluded. In addition, welfare measures (hock burn, footpad dermatitis, and feather cleanliness) were scored at 33d, prior to moving birds into pens. Gait and welfare measures were again scored at 37d. Welfare measures were compared among conditions and time points using PROC LOGISTIC (SAS 9.4) to calculate odds ratios. Odds ratios determine the likelihood of one group/age having a score of 0 vs. the other group/age. Walking ability decreased in all groups from 33d-37d. At 37 d, 78% of S birds, 17% of P birds, 49% of MF birds and 29% of ML birds experienced decreased walking ability. All groups experienced a decrease in other welfare measures; odds ratios indicated that all groups had low odds of having no hock burn, no footpad dermatitis, and clean feathers at 37d ($p < 0.0001$). The P, ML, and MF birds had lower odds of having a 0 gait score at 37d vs. 33 d ($p < 0.0001$). By 37d, ML birds were more likely to have clean feathers compared to all other groups ($p < 0.0001$), and S birds were more likely to have clean feathers compared to P ($p < 0.0001$) and MF ($p < 0.0001$) birds. In addition, P birds were less likely to have no hock burn compared to S birds ($p < 0.0001$), MF birds ($p < 0.0001$), and ML birds ($p < 0.0001$). These results indicated that gait of all birds declined with age, but less so in birds housed at the lower stocking density. Therefore, walking ability, stocking density, and age influence the welfare of broiler chickens.

Key Words: Broiler; Welfare ; Gait; Stocking Density

27 Effect of pasture type on range use in fast-growing broiler chickens. Bidur Paneru^{1*}, Gabriel Pent¹, Shawna Nastasi¹, Adam Downing¹, John Munsell¹, John Fike¹, Leonie Jacobs¹, ¹Virginia Tech, Blacksburg, Virginia, United States.

Access to an outdoor range provides opportunities for exercise in broiler chickens and has been positively associated with animal welfare outcomes such as improved leg strength and gait compared to broilers housed solely

indoors. USDA-certified organic production requires outdoor access for chickens but provides no or limited natural vegetation other than grass. These conditions do not provide opportunities to seek cover from predators or avoid extreme weather conditions, contributing to limited range use. Providing access to silvopasture, an area with larger vegetation for shelter that may also serve as an additional source of income (fiber, fruit, nuts, woody decoratives, etc.) for the producer, may benefit ranging behavior. The study objective was to evaluate the effect of pasture type (silvopasture; SP or open pasture; OP) on range use in fast-growing broiler chickens. Day-old mixed-sex Ross 708 chicks (n=864) were housed in 12 pens (53/54 birds per pen) until day (d) 22 and were provided access to range (six 125m² SP plots; average mean=32% canopy cover or six OP plots; without canopy cover) from d23. Plot-level range use (% of the flock outside) was assessed hourly from photographs (wildlife cameras) from d24-41 (890-899 photos per treatment) between 8 AM and 5 PM. Range use data were analyzed with mixed models in JMP Pro 16 with treatment, age (week), and their interactions as fixed factors, tested by time of day (morning, midday, afternoon), with plot ID as a random factor. Non-significant interactions (P<0.1) were removed from the model. Data are presented as LSMEAN±SE. The SP and OP ranges were equally used in the mornings regardless of treatment (SP:12.5±0.9% vs. OP:12.8±1.0%; p=0.926), yet fewer birds were ranging in the mornings in week 3 (1.6±2.7%) compared to week 5 (17.0±1.0%) and 6 (17.7±1.5%), in week 4 (7.4±1.0%) compared to week 5 and 6, and in week 5 compared to week 6 (all Tukey HSD p<0.001). At midday, more birds ranged in SP than in OP treatments (SP:1.0±0.4% vs. OP:0.0±0.4%; p=0.034). Midday range use was not impacted by age (p=0.575). In the afternoon, range use was impacted by a treatment by age interaction (p=0.010), with most birds ranging in SP in week 5 (7.2±0.9%) compared to all other treatment and age combinations (all p≤0.006). Our findings show a preference for morning range use regardless of range type. In addition, providing fast-growing broiler chickens access to a silvopasture will improve range use compared to an open grass pasture, especially in the afternoon, with range use peaking at 5 weeks of age.

Key Words: animal welfare; behavior; silvopasture; outdoor access; time of day

28 Effects of environmental enrichment on turkey welfare and walking ability. Yiru Dong^{1*}, Gregory Fraley¹, Janice Siegford², Fengqing Zhu¹, Marisa Erasmus¹, ¹Purdue University, West Lafayette, Indiana, United States, ²Michigan State University, East Lansing, Michigan, United States.

Environmental enrichment (EE) is one effective way to reduce injurious pecking and improve poultry welfare. However, little is known about the age-related benefits of EE to improve the walking ability and welfare of commercial tom turkeys. This study investigated age-

related changes in turkey welfare measures (wounds, feather quality (FQ), feather cleanliness and footpad condition) and walking ability (gait) as influenced by five different types of EE. Turkeys (n = 420) were randomly assigned to one of six treatment groups: a straw bale (S), platform (P), platform + straw bale (PS), pecking block (B), or tunnel (T) as enrichment or to a control group (C; no enrichment). Welfare measures and gait were assessed at 8, 12, 16 and 19 wk and analyzed using PROC LOGISTIC with Firth's bias-correction (SAS 9.4). Age significantly affected most welfare measures and gait, while minimal overall treatment effects were observed. Better wing FQ with age was observed in turkeys in S and T groups. Compared to 8 wk of age, turkeys in the S group had better wing FQ at 16 (P = 0.028) and 19 wk (P = 0.011). Similarly, wing FQ (P = 0.008) was better at 19 wk vs. 8 wk for T turkeys. Unlike FQ that improved with age in some cases, footpad condition worsened over time for turkeys in all treatment groups except for the S group. Footpad condition was worse at 19 wk than at 8 wk for turkeys in P (P = 0.024), PS (P = 0.039), B (P = 0.011), T (P = 0.004) and C (P = 0.014) groups and was worse at 19 than at 12 wk for turkeys in B (P = 0.038), T (P = 0.015) and C (P = 0.045) groups. Footpad condition was worse at 19 than at 16 wk for turkeys in the T (P = 0.007) and C (P = 0.048) groups. Footpad condition was also worse at 16 wk than at 8 wk for turkeys in the B (P = 0.046) group. Gait also worsened with increasing age in all treatment groups. Gait was worse at 19 wk for S (P < 0.001), P (P < 0.001), PS (P < 0.001) and B turkeys (P < 0.001) compared to 8, 12 and 16 wk, while gait in T (P < 0.001) and C turkeys (P < 0.001) worsened starting at 16 wk. In conclusion, providing turkeys with a straw bale and tunnel can help improve wing feather quality with age, and providing straw bales can reduce the development of footpad dermatitis. Providing elevated structures (bale or platform) and EE that satisfies turkeys' pecking and foraging needs (straw bale or pecking block) can potentially delay the onset of gait problems compared to turkeys not provided with enrichment or provided with a tunnel.

Key Words: gait; turkey; welfare; environmental enrichment; walking ability

29 Two are better than one?: Effects of Environmental Enrichments Combinations on Well-being measures in colony caged Japanese Quail (*Coturnix japonica*). Chirantana Mathkari^{1*}, Rachel Dennis¹, ¹University of Maryland, College Park, Hyattsville, Maryland, United States.

Breeding purpose Japanese quail (*Coturnix japonica*) are often maintained in barren cages with little stimulation. These conditions commonly show stress-related behaviors that can cause serious injury and mortality. Environmental enrichments (EEs) that can provide protection from unwanted interactions (e.g. protective hut), or that can provide mental stimulation (e.g. mirror) are potential solutions to this welfare issue. Our previous research

showed that adding either type of EE to the birds' cage reduces stress-related behaviors and injuries, without impacting their productivity. The current study aimed at evaluating the effectiveness of simultaneous exposure to 2 such EEs on breeding quail well-being. 29 breeding colony cages of quail (1 male, 2 females/cage) were used. The treatments consisted of: protective hut with octagonal mirror (H+OM), protective hut with rubber mat (H+RM), and protective hut with feeder toy (H+FT). Using an incomplete Latin square design, each cage received one of the 3 EE combinations or a control (protective hut) for a period of four weeks (n=13/trt). Behaviors and production parameters were analyzed using a one-way ANOVA; and body scores were analyzed using a Chi-square in SAS 9.4. H+RM was the most interacted EE combination ($P<0.001$). No notable interaction differences were seen between H+FT and H+OM ($P>0.05$). Interactions with the hut were significantly more in the H+OM birds than those in the H+RM, H+FT and control groups ($P<0.0001$, $P=0.007$ and $P=0.027$). Compared to the control, cage pecking reduced significantly in H+RM and H+FT ($P<0.0001$ and $P=0.049$).

Vigilant behavior was significantly decreased in H+OM than in the control ($P=0.018$). Sexual mounting in the treatments did not differ from the control ($P>0.05$). The treatments did not impact bird pecking or preening frequencies ($P>0.05$). Compared to the control, H+OM exhibited a trend of reduced walking and increased standing ($P=0.050$ and $P=0.066$). Birds in the H+RM group sat notably more than those in the control ($P=0.024$). The treatments did not impact eating and drinking behaviors ($P>0.05$). Birds' body weight, egg production, injury scores and footpad scores remained unaffected by the treatments ($P>0.05$). Compared to control, H+OM and H+FT showed a trend of improved feather score ($P=0.067$). Overall, exposure to hut along with another EE reduced more stress-related behaviors than exposure to only hut. Our results suggest that compared to a single EE, the use of optimal EE combinations in breeding quail can mitigate a larger variety of stress-related behaviors and improve well-being.

Key Words: Quail; Welfare; Behavior; Housing; Environmental enrichment

Student Competition: Processing and Products

30 Evaluation of forming agent inclusion on textural profile analysis of dehydrated pet treats with different thickness manufactured from broiler chicken wing tips.

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Wingtips (WT) are co-products resulting from broiler chicken processing. Due to their low consumer acceptance and market price, WT may be used by pet treats manufacturers as an alternative protein source. Inclusion of a structure forming agent (ALGIN) containing sodium alginate (SA) and encapsulated calcium lactate (ECL) can aid in the formation of pet treats derived from broiler processing co-products. The purpose of this study was to evaluate texture profile analysis (TPA) of pet treats generated from WT with 4 ALGIN inclusions sliced to 2 different thicknesses. Previously frozen WT were ground through a 4.8-mm grinder plate and mixed with 1 of 4 ALGIN concentrations: a control (0X) with no added ALGIN, 0.5X (0.425% ECL + 0.5% SA), 1X represents the manufacturers recommended inclusion (0.85% ECL + 1% SA), and 2X (1.7% ECL + 2.0% SA). Mixtures were extruded into 63.5-mm-diameter casings and covered with plastic wrap. Chubs were refrigerated at 4 °C for 12 h to allow gelation and then frozen at -20 °C for 3 h to facilitate slicing into 5 and 10-mm-thick slices to produce 8 treatments. Treats were dehydrated at 93.3 °C until an internal temperature of 73.8 °C was reached and then at 65.6 °C until water activity reached 0.8. A TA-HDplusC texture analyzer with a TA-30-cylinder probe was used to evaluate hardness, resilience, cohesiveness, chewiness, and springiness on 10 samples per variable per treatment of dehydrated slices using 2-cycle compression. Data were analyzed as a 2-way ANOVA using the GLIMMIX procedure of SAS and means were separated at $P \leq 0.05$. No ALGIN \times slice thickness interactions were observed for hardness (force required to deform), resilience (ability to regain original form after compression), cohesiveness (structural integrity under compression), and chewiness (energy required to masticate; $P \geq 0.1391$). No differences were observed for hardness ($P \geq 0.3657$). The 10-mm-thick slices had greater ability to regain their original form after compression ($P \leq 0.0001$), kept major structural integrity under compression ($P \leq 0.0001$), and more energy was required to masticate ($P = 0.0002$). An ALGIN \times slice thickness interaction was observed for springiness and 10-mm-thick treats containing 0.5X ALGIN recovered the most height after compression among all treatments ($P = 0.0308$). In conclusion, increasing slice thickness resulted in treats with greater resilience, cohesiveness, and chewiness.

Further characterization will be required to determine the acceptability by pets and pet owners of these treats manufactured using structure forming technology and low-value WT.

Key Words: Dehydration; Texture; Co-product; Sodium alginate

31 Evaluation of forming agent inclusion and slice thickness on textural profile analysis of raw pet treats generated from broiler wing tips.

Cristopher Almendares^{1*}, Jorge Romero¹, Justin Dunavant¹, Diego Ventura Urbina¹, Josue Cerritos¹, Alexandra Caballero¹, Hilary Carrera¹, Hector Fajardo Menjivar¹, Ileana Berganza¹, Juan Barberena¹, Luis Guzman¹, Francisco Castilblanco¹, Eric Altom², Jessica Starkey¹, Charles Starkey¹, ¹Auburn University, Auburn, Alabama, United States, ²Balchem Corp., New Hampton, New York, United States.

Poultry processing generates co-products with reduced commercial value. Wing tips (WT) are the most distal portion of the wing. Forming agents are often used in ground meat-containing products to provide structure. ALGIN is the combination of sodium alginate (SA) and encapsulated calcium lactate (ECL). Characteristics, such as texture, can determine the quality of a meat product. The objective was to evaluate the effect of different ALGIN inclusions and slice thickness on texture profile analysis (TPA) of raw pet treats. Previously frozen WT were ground through a 4.8-mm diameter grinder plate and mixed with 1 of 4 different ALGIN inclusions: 0X (no ALGIN inclusion), 0.5X (0.425% ECL + 0.50% SA), 1X (0.85% ECL + 1.00% SA; manufacturer's recommended inclusion), and 2X (1.70% ECL + 2.00% SA). After mixing, each treatment was extruded into 63.5-mm diameter casings, covered with plastic wrap, refrigerated at 4 °C for 12 h, and frozen at -20 °C for 3 h prior slicing. Chubs were cut into 5 and 10-mm-thick slices for texture analysis. Hardness (maximum force required to deform), adhesiveness (adhesion of sample to the probe), resilience (ability of sample to regain original form), cohesiveness (represents structural integrity under compression), springiness (height the food recovers between compressions), and chewiness (energy required to masticate) were measured on 10 samples per treatment using a TA-30-cylinder probe and 2-cycle compression. Data were analyzed as a 2-way ANOVA using the GLIMMIX procedure of SAS and least square means were separated using the PDIF option at $P \leq 0.05$. No ALGIN \times slice thickness interactions were observed ($P \geq 0.0924$). As ALGIN inclusion increased from 0X to 1X, raw treats required greater force to be deformed (hardness; $P = 0.0006$). Neither ALGIN or slice thickness affected raw treat adhesiveness or resilience ($P \geq 0.0556$). Raw treats with 0.5X ALGIN exhibited the greatest structural integrity under compression (cohesiveness; $P = 0.0336$). Raw samples containing 0.5X and 2X ALGIN recovered more

height between compressions (springiness; $P = 0.0208$) and required more energy to masticate (chewiness; $P \leq 0.0001$) compared with those containing 0X and 1X ALGIN. Raw treats cut to 10-mm had greater cohesiveness, springiness, and chewiness but lower hardness values compared with those cut to 5-mm-thick ($P < 0.0001$). In conclusion, slice thickness did not interact with ALGIN inclusion to alter TPA characteristics of raw pet treats derived from upcycled broiler WT. Further investigation will be required to understand the impact of these changes in raw treat TPA characteristics on pet and pet owner acceptance and purchasing decisions.

Key Words: textural characteristics; pet treats; sodium alginate; encapsulated calcium lactate; wing tip upcycling

32 Effect of slice thickness and forming agent inclusion on textural characteristics of raw pet treats derived from broiler wing tips. Hilary Carrera^{1*}, Jorge Romero¹, Justin Dunavant¹, Diego Ventura Urbina¹, Josue Cerritos¹, Alexandra Caballero¹, Cristopher Almendares¹, Hector Fajardo Menjivar¹, Ileana Berganza¹, Juan Barberena¹, Luis Guzman¹, Francisco Castilblanco¹, Eric Altom², Jessica Starkey¹, Charles Starkey¹, ¹*Auburn University, Auburn, Alabama, United States*, ²*Balchem Corp., New Hampton, New York, United States*.

The broiler chicken industry generates many low-value co-products. Chicken wing tips (WT) are the most distal section of the wing and are not commonly desirable for human consumption. Broiler processing co-products, such as WT, may be upcycled by the pet food industry as they may be useful in the design of pet treats. The gelling of meat products can be improved by inclusion of a structure agent forming ALGIN, which is the combination of sodium alginate (SA) and encapsulated calcium lactate (ECL). The objective of the experiment was to evaluate the effect of different ALGIN inclusions and slice thickness on textural characteristics of raw pet treats. Previously frozen, WT were ground through a 4.8-mm grinder plate and mixed with 1 of 4 different ALGIN inclusions: 0X (no ALGIN inclusion), 0.5X (0.425% ECL + 0.50% SA), 1X (0.85% ECL + 1.00% SA; manufacturer's recommended inclusion), and 2X (2.00% ECL + 1.70% SA). After mixing, product was extruded into 63.5-mm-diameter casings, wrapped in plastic wrap, refrigerated at 4 °C for 12 h, and frozen at -20 °C for 3 h prior to slicing. Chubs were sliced into 5 and 10-mm thick slices to simulate commercially available products. The 3-point-bend (3PB) and shear force tests were conducted on samples ($n = 10$ samples per treatment per analysis) using a TA-HDplusC texture analyzer with a TA-43R and TA-42 probe, respectively. The 3PB test assesses hardness (force to break), flexibility (distance to break), and stiffness (force/distance). Shear force evaluates firmness (peak force to break) and toughness (total force over distance). Data were analyzed as a 2-way ANOVA using the GLIMMIX procedure of SAS with mean separation at $P \leq 0.05$ using the PDIFF option. Raw 5-mm-thick treats with

0X ALGIN were the stiffest compared with those from all other treatments ($P = 0.003$). No ALGIN \times slice thickness interaction was observed for hardness, flexibility, and firmness ($P \geq 0.0517$). Raw treats sliced to 10-mm were able to bend further before breaking ($P < 0.0001$) and treats with 0X ALGIN were the least flexible ($P < 0.0001$). Thinner raw slices (5-mm-thick) were firmer ($P = 0.0417$), but similar in hardness ($P = 0.3305$) compared with 10-mm-thick slices. Finally, 10-mm-thick raw treats containing 2X ALGIN were the toughest when compared to all other treatments ($P = 0.0011$). In conclusion, ALGIN inclusion and treat slice thickness both impacted textural characteristics of raw WT-derived pet treats. Though, it is unclear how these alterations will impact pet and pet owner acceptability and buying decisions.

Key Words: hydrocolloids; broiler chicken processing; texture analysis; pet treats; wing tip

33 Effect of forming agent inclusion and slice thickness on textural characteristics of dehydrated pet treats derived from broiler chicken wing tips. Ileana Berganza^{1*}, Jorge Romero¹, Justin Dunavant¹, Cristopher Almendares¹, Diego Ventura Urbina¹, Josue Cerritos¹, Alexandra Caballero¹, Hector Fajardo Menjivar¹, Juan Barberena¹, Hilary Carrera¹, Luis Guzman¹, Francisco Castilblanco¹, Eric Altom², Jessica Starkey¹, Charles Starkey¹, ¹*Auburn University, Auburn, Alabama, United States*, ²*Balchem Corp., New Hampton, New York, United States*.

Broiler chicken processing results in the generation of various low-value co-products that are high quality protein sources. Chicken wing tips (WT) are the most distal part of the wing and may be useful in the design of pet treats to improve value. Addition of structure forming technologies such as ALGIN which is composed of sodium alginate (SA) and encapsulated calcium lactate (ECL) can aid in structural stability of meat products. The objective of this study was to assess the effect of different ALGIN inclusions on textural characteristics of dehydrated WT derived pet treats cut to 1 of 2 different commercially available thicknesses. Previously frozen WT were ground with a 4.8-mm diameter grinder plate. ALGIN was added and mixed with ground WT at 1 of 4 concentrations: 0.5X (0.50% SL + 0.425% ECL), 1X (1.00% SL + 0.85% ECL), and 2X (2.00% SL + 1.70% ECL) the manufacturer's recommended inclusion plus a control group with no ALGIN inclusion (0X). Product was then extruded into 63.5-mm diameter casings, wrapped with plastic film, refrigerated at 4°C for 12 h, and frozen at -20°C for 3 h prior to slicing. Chubs were sliced into 5 and 10-mm-thick discs, dehydrated at 93.3 °C until an internal temperature of 73.8 °C was reached, and then at 65.6 °C until the samples reached 0.8 aW ($n = 10$ samples from each treatment per analysis). A TA-43R probe on a TA-HDplusC texture analyzer was used for the 3-point bend test to assess: hardness (force to break), flexibility (distance to break), and stiffness (force over distance), while

a TA-42 probe was used for the shear force test to evaluate firmness (peak force) and toughness (total force over distance). Data were analyzed as a 2-way ANOVA using the GLIMMIX procedure of SAS with mean separation at $P \leq 0.05$. Flexibility and firmness values of 5-mm-thick treats were lower compared with 10-mm-thick treats without ALGIN ($P = 0.0034$) but treats with 2X ALGIN had similar flexibility among thickness. Ten-mm-thick treats with 0.5X, 1X, and 2X ALGIN inclusion were the stiffest among all samples ($P = 0.0005$). No ALGIN \times slice thickness interaction was observed for toughness ($P = 0.1068$). However, 10-mm treats were tougher than 5-mm treats ($P < 0.0001$) and treats with 2X ALGIN inclusion required the greatest force over the distance to slice the sample ($P < 0.0001$). Force required to break 10-mm treats with 2X ALGIN inclusion was greater compared with 5-mm treats with 0.5X, 1X, and 2X ALGIN ($P < 0.0001$). Increasing ALGIN inclusion above 1X and slice thickness to 10-mm produced less flexible, tougher, and harder WT-derived pet treats, though it is unclear how this might affect pet owner buying decisions.

Key Words: alginate; texture analysis; broiler processing co-products; chicken wing tips

34 Comparison of textural profile analysis of raw pet treats manufactured with wing tips and structure forming technology with increasing degrees of probe strain. Said Herrera^{1*}, Jorge Romero¹, Justin Dunavant¹, J. Wesley Rogers¹, Diego Ventura Urbina¹, Haisten Smith¹, Joshua Renew¹, Jorge Banegas¹, Hilary Carrera¹, Cristopher Almendares¹, Luis Guzman¹, Clauber Polese¹, Alexandra Caballero¹, Eric Altom², Jessica Starkey¹, Charles Starkey¹, ¹*Auburn University, Auburn, Alabama, United States*, ²*Balchem Corp., New Hampton, New York, United States*.

Broiler chicken wing tips (WT) are broiler processing co-products that are often undervalued and used for purposes other than human consumption like being incorporated into pet food. Structuring forming agents have the potential to transform WT into pet treats. Textural characteristics of pet treats may influence a purchasing decision. The objective was to compare texture profile analysis (TPA) of raw pet treats generated from WT blends with a structure forming agent inclusion (ALGIN = sodium alginate (SA) and encapsulated calcium lactate (ECL)) using 1 of 3 different degrees of probe strain: 20, 35, or 50% compression relative to the treat's original thickness. Previously frozen WT were ground through a 4.8-mm grinder plate and mixed with either ALGIN (2.0% SA + 1.70% ECL) or without ALGIN to produce treats with different texture profiles. Mixtures were extruded into 63.5-mm-diameter casings, stored overnight at 4 °C, frozen at -20 °C for 3 h to allow gelation and facilitate slicing into 5-mm-thick discs for TPA to simulate commercially available products. Thirty samples per treatment per probe strain setting were evaluated using a TA-HDplusC texture analyzer with 2-cycle compression to obtain values for hardness (maximum force required to

deform), adhesiveness (force to separate from probe), resilience (ability of sample to regain original form), cohesiveness (structural integrity under compression), springiness (proportion of height the sample recovers after mastication), and chewiness (energy required to masticate). Data were analyzed as a 2-way ANOVA with the GLIMMIX procedure of SAS and means were separated at $P \leq 0.05$ using the PDIF option. An ALGIN \times probe strain interaction was observed for all variables measured ($P \leq 0.0046$). Hardness increased as probe strain and ALGIN increased ($P = 0.0005$). The adhesion of the sample to the probe decreased with ALGIN inclusion when probe strain was 35 and 50% of the original sample thickness ($P < 0.0001$). Treat resilience increased with ALGIN inclusion and when raw treats were compressed 20 and 35% of their original thickness ($P = 0.0046$). As expected, the hardness, resilience, cohesiveness, springiness, and chewiness of raw treats derived from WT increased with ALGIN inclusion ($P < 0.0001$). Differences in adhesiveness ($P = 0.0561$) and resilience ($P = 0.1859$) among treats with and without ALGIN were not detected with probe strain settings of 20% and 50%, respectively. Therefore, based on the ability to detect differences in all TPA textural characteristics among treats with different ALGIN inclusions, using a probe strain setting of 35% for TPA appears to be most appropriate for raw, bone-in, WT-derived pet treats.

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

35 Effect of structure forming agent concentration and thickness of raw pet treats derived from broiler chicken wing tips on instrumental color over time post-production. Hector Fajardo Menjivar^{1*}, Cristopher Almendares¹, Justin Dunavant¹, Jorge Romero¹, J. Wesley Rogers¹, Marc Presume¹, Said Herrera¹, Joshua Renew¹, Jorge Banegas¹, Gerardo Abascal-Ponciano¹, Diego Ventura Urbina¹, Luis Guzman¹, Tristan Reyes¹, Madison Wagoner¹, Robert Mason², Eric Altom², Jason Sawyer¹, Jessica Starkey¹, Charles Starkey², ¹*Auburn University, Auburn, Alabama, United States*, ²*Balchem Corp., New Hampton, New York, United States*.

Broiler chicken wing tips (WT) are a low-value broiler processing co-product with potential for conversion into pet treats. Pet owner purchasing decisions can be influenced by product color and changes in color post-production. The objective was to evaluate instrumental color over time of pet treats generated from raw, ground WT containing different inclusions of a structure forming agent (ALGIN) composed of sodium alginate (SA) and encapsulated calcium lactate (ECL) and being sliced at 2 different commercially available thicknesses. Previously frozen, chicken WT were ground through a 4.8-mm grinder plate and mixed with 1 of 4 ALGIN concentrations: a control (0X) without ALGIN, 0.5X (0.5% SA and 0.425 ECL), 1X (1% SA and 0.85% ECL; manufacturer's recommended inclusion), and 2X (2% SA and 1.70% ECL). Product was extruded

into 63.5-mm-diameter casings, stored overnight at 4 °C, and frozen at -20 °C prior to slicing into either 5 or 10-mm-thick samples. A Hunter Lab Miniscan XE Plus colorimeter was used on a white background to measure surface color (CIE L* (lightness), a* (redness), b* (yellowness)) in triplicate on each treat (n = 10 samples per treatment) after 0, 3, 5, and 7 d of storage post-slicing at 4 °C. Data were analyzed as a 3-way ANOVA with the GLIMMIX procedure of SAS and means were separated at $P \leq 0.05$ using PDIF. Regardless of ALGIN inclusion or slice thickness, raw treats were lighter on d 7 compared with d 0, 3, and 5 post-production ($P < 0.0001$). On d 7, raw treats sliced to 10-mm containing 0X and 0.5X ALGIN had the highest L* values, whereas on d 0, 10-mm-thick treats containing 1X and 2X ALGIN had the lowest L* values ($P < 0.0001$). Regardless of ALGIN inclusion or slice thickness, raw treats had greater a* values on d 0 than on d 3, 5, and 7 ($P < 0.0001$). Treats sliced to 10-mm containing 1X and 2X ALGIN had the highest a* values ($P < 0.0001$). After d 0, regardless of ALGIN inclusion or slice thickness, b* values decreased except for 10-mm-thick treats without ALGIN ($P < 0.0001$). Regardless of ALGIN inclusion or slice thickness, raw treat b* values increased on d 7 except for 5 and 10-mm-thick treats containing 2X ALGIN ($P < 0.0001$). In conclusion, the instrumental color of raw, WT-derived, pet treats was differentially affected by ALGIN inclusion, slice thickness, and refrigerated storage time post-production. However, it is unclear how this may influence pet owner buying preferences or if the pet owner can visually detect the differences in color observed in this study with WT-derived raw pet treats.

Key Words: broiler chicken processing; pet treats; color variation post-production; instrumental color; structure forming technology

36 Evaluation of stress hormone indicators in broiler chickens stunned by either electrical or controlled atmosphere stunning methods under commercial conditions. Montana Riggs¹*, Ruediger Hauck¹, Bethany Baker-Cook¹, Rachel Osborne¹, Amrit Pal¹, Maria Tereza Bethonico Terra¹, Gracie Sims¹, Andrea Urrutia¹, Leticia Orellana Galindo¹, Marco Reina Antillon¹, Dianna Bourassa¹, ¹Auburn University, Auburn, Alabama, United States.

Increased consumer concern of animal welfare has led some poultry producers to alter their stunning methods from electrical to controlled atmosphere stunning. Controlled atmosphere stunning has been suggested to be more humane than electrical stunning, which should lead to a lower level of hormonal indicators of a stress response. The objective of this study was to assess the impact of electrical stunning (ES) or controlled atmosphere stunning (CAS) on the levels of physiological stress hormones of broilers at a commercial facility. Broilers were processed at a body weight averaging 2.04 kg per bird. Blood was collected from 30 broilers (15 ES, 15 CAS) at lairage, 60 broilers (30 ES and 30 CAS) immediately pre-stun and 60 broilers (30 ES and 30 CAS)

post-stun. For pre-stun, each bird was removed from the process immediately prior to entering the waterbath or stunning chamber. Post-stun broilers were removed from the process within 15 s following stunner exit. Blood was collected for each bird immediately upon removal from the process. Corticosterone (CORT), adrenocorticotropic hormone (ACTH), epinephrine (EPI), and norepinephrine (NORE) were measured by ELISA. Data were analyzed using generalized linear models with a significance at $P \leq 0.05$. Means were separated by Tukey's HSD. CORT concentrations (pg/mL) were not changed over time for either ES (73 at lairage, 84 pre-stun, 63 post-stun, $P=0.1607$) or CAS (66 at lairage, 51 pre-stun, 51 post-stun, $P=0.2113$). ACTH concentrations (ng/mL) were not changed over time for either ES (719 at lairage, 538 pre-stun, 613 post-stun, $P=0.1240$) or CAS (176 at lairage, 127 pre-stun, 109 post-stun, $P=0.0556$). EPI concentrations (pg/mL) for ES post-stun (1.55) were higher than immediately pre-stun (1.09), but not different than at lairage (1.28, $P=0.0317$). For CAS broilers EPI concentrations were not different over time (2.04 at lairage, 1.98 pre-stun, 1.88 post-stun, $P=0.5262$). NORE concentrations (pg/mL) were not changed over time for either ES (20.50 at lairage, 25.57 pre-stun, 25.60 post-stun, $P=0.7976$) or CAS (24.10 at lairage, 21.92 pre-stun, 17.10 post-stun, $P=0.1686$). These results indicate that stunning broilers using either ES or CAS in a commercial facility does not lead to increases in CORT, ACTH, EPI, or NORE. Based on the blood hormone indicators that were measured, both ES and CAS were equally acceptable.

Key Words: corticosterone; poultry; stunning; adrenocorticotropic hormone; epinephrine

37 Rate of blood loss and total blood loss varies in broilers based on exsanguination method. Rachel Osborne¹*, Caitlin Harris¹, R. Buhr², Brian Kiepper¹, ¹University of Georgia, Athens, Georgia, United States, ²USDA ARS, Athens, Georgia, United States.

With over 9 billion broilers processed each year in the U.S., blood is a significant and valuable by-product of poultry processing. Yet, there is relatively little research into the effect of exsanguination method on overall blood loss and blood loss rate, particularly on modern high breast meat yield broilers. It has been assumed that decapitation may result in a less effective bleed-out than conventional neck-bleed cuts. However, there are no definitive studies available examining this issue with modern-day, feed withdrawn broilers. Without access to accurate bleed-out data, processors may have difficulty deciding on an appropriate bleed-out time and corresponding blood collection tunnel length. The aim of this study was to determine percentage of blood loss and rate of blood loss for 4 exsanguination methods. To accomplish this, 4 trials were conducted using male broilers at 51d (N=88; BW 3896g), 45d (N=96; BW 3923g), 44d (N=88; BW 3681g), and 36d (N=80; BW 2480g) of age. Broilers underwent an

8-hour feed withdrawal before being shackled, then electrically stunned (25V pulsed DC for 15s, mouth to vent) and randomly assigned to be exsanguinated in one of 4 ways: neck cut (carotid artery and jugular vein) on one side (1S), neck cut on both sides (2S), decapitated at the base of the head (DH), or decapitated at the base of the neck (DN) using a hand-held knife. Post exsanguination, BW were recorded at 15s intervals until 3 minutes had elapsed. Rate of blood loss and percentage total blood loss were calculated based on BW loss, and data were analyzed by ANOVA in SAS JMP using Student's t-test for means separation. 1S treatment consistently resulted in the highest percentage total blood loss across all trials (3.01%, 51d; 3.80%, 45d; 3.44%, 44d; 3.89%, 36d), while DN resulted in the lowest percentage total blood loss in all trials (2.69%, 51d; 3.47%, 45d; and 3.53%, 36d), except for birds 44d of age (2.99%). As bird age and BW increased, blood lost as a percentage BW over 180s tended to decrease. Regardless of bird age, the highest rate of blood loss occurred in the first 15s after exsanguination and significant differences in blood loss rate were greatly reduced after 75s of bleed-out time. 1S had a significantly lower rate of blood loss in the first 15s in birds 51d of age ($P < 0.0001$), 45d of age ($P = 0.0011$), and 36d of age ($P < 0.0001$). 73% (51d) to 77% (44d) of all blood lost during bleed-out occurred in the first 30s. After 90s, only 5% (36d) to 7% (51d) of total blood loss was recorded. This indicates that while overall blood loss may vary based on exsanguination method, a sufficient bleed-out can be achieved within 90s (current industry standard) regardless of broiler age or BW.

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

38 Performance and processing characteristics of male Ross 708 broilers supplemented with guanidinoacetic acid. Clay Maynard¹*, David Nelson², Samuel Rochell¹, Casey Owens¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Alzchem, LLC, Trostberg, Germany.

Performance advantages have been noted in meat animal production when excess creatine is supplied in the diet. In the current experiment, 750 male day of hatch Ross 708 broilers were placed 25 per pen and reared for 56 days over 10 replicate pens per treatment. Dietary treatments were established using a control basal diet fed over four feeding phases with 1.32, 1.21, 1.10, and 1.06% digestible lysine for each phase, respectively. Treatments 1 and 2 were then allocated as an additional supplementation of 0.06% (T₁) and 0.12% (T₂) guanidinoacetic acid (GAA) in the form of CreAMINO (Alzchem Group, Trostberg, Germany). Bi-weekly BW, BWG, FI, and mortality corrected FCR were calculated throughout the duration of the growing phase. Eighteen birds per pen were processed and cut up into boneless breast and tender, wing, and whole leg parts to calculate yields. Breast and tenders were assessed for myopathies including woody breast, white striping, woody-

like tender, and tender feathering (fraying of the *Pectoralis minor*). Data were analyzed as a one-way ANOVA testing the main effect of GAA supplementation. For the overall growout period, broilers fed either T₁ or T₂ consumed less feed than the control diet (lower feed intake; $P < 0.05$). Treatment 1 and 2 improved FCR ($P < 0.05$) when compared to the control diet at 55d. No differences ($P > 0.05$) were observed for BW or BWG throughout the growout period. At day of processing, control broilers expressed heavier live weights than T₁ and T₂ ($P < 0.05$; 4808g vs 4684g and 4657g, respectively), but no differences were observed in yields ($P > 0.05$). For woody breast and woody-like tender, the control produced a higher degree of severity ($P < 0.05$) when compared to T₂. For white striping, the control and T₁ expressed a higher severity ($P < 0.05$) over that observed in T₂. No differences were observed in tender feathering ($P > 0.05$). The results of this experiment indicate that GAA supplementation in broiler diets may be an effective tool for improving meat quality by reducing myopathy severity, without negatively affecting growth performance.

Key Words: broiler; guanidinoacetic acid; performance; white striping; woody breast

39 Effect of Dietary Micro Algae Supplementation on Broiler Chicken Carcass and Meat Quality. Jasmine Moallem¹*, ¹California Polytechnic State University-San Luis Obispo, San Luis Obispo, California, United States.

The study was performed to evaluate the effect of dietary microalgae on broiler feeding for carcass weight and meat quality. A total of 360 chicks were randomly allocated to 24 floor pens (15 birds/pen, 8 pens/treatment) and fed up to 58 days with one of three dietary treatments of 0% (control), 2.5%, and 5% microalgae (*Uronema* spp.) meals. Dietary treatments were prepared with isonitrogenous and isoenergetic practices for the corn-SBM diet and the three microalgae (*Uronema* sp.) diets. On day 59, 24 chickens were randomly selected from 24 pens (one chicken/pen) and conventionally processed. After chilling, breast fillets were harvested to measure pH, sarcomere length, and meat tenderness using the method of the Meullenet Owens Razor Shear (MORS). Instrumental color (L*, a*, and b*) on broiler breasts was measured with/without skin. The experiment was completely randomized, and all data were analyzed using the PROC MIXED procedure of SAS. Broilers treated with 5% algae showed heavier carcass weight than control ($P < 0.05$). Algae supplement level did not affect carcass chilling yield, meat pH, and cooking yield ($P > 0.05$). However, breast fillets from the broilers with 5% algae had lower shear than those of control and 2.5 % algae ($P < 0.05$), with no sarcomere length difference regardless of treatment. A stepwise increase of yellowness was found in the breast muscles from 0% to 5% algae although no difference was observed for lightness and redness regardless of skin presence. Based on these results, feeding broiler chickens with 5% algae may result in positive effects on

broiler live weight, carcass weight, and meat tenderness with no other meat quality loss.

Key Words: Carcass quality; Algae feeding; Broiler processing; Carcass color; Meat tenderness

40 Reducing processing metric variation for nutrition research. Caleb Marshall^{1*}, Nickki Tillman², Kelley Wamsley¹, ¹*Mississippi State University, Mississippi State, Mississippi, United States*, ²*Nutritional Statistics LLC, Buford, Georgia, United States*.

Experimental designs are carefully considered to control for variables outside of the scope of a given study; however, the focus to reduce variation for nutrition research is often given to live performance metrics before processing metrics. Nutrition research notoriously relies on being able to determine small differences between dietary treatments (TRT) effects, which can be masked by variation. Since processing data are a vital component to determine if certain nutrition interventions that add costs to the overall diet are worth the investment, the objective of the current study was to evaluate processing personnel (PP) variation and use PP as a covariant for analysis of nutrition TRT effect from 3 nutrition experiments (EXP) on processing cut-up metrics (EXP 1, 2- 6 PP; EXP 3- 5 PP). All EXP utilized Ross x Ross 708 male broilers and processed 5 birds/pen of average weight (± 100 g or closest to that); 5 birds/pen were chosen as it is a common number for nutrition research. Processing and cut-up occurred for each EXP (1- d 43, n=320; 2- d 57, n=320; 3- d 56, n=240). Tagged carcasses were randomly assigned to PP, and each PP was assigned a basket color;

parts (with tag) were placed in corresponding baskets for both part weights and PP to be recorded for each carcass. The same PP were utilized for each EXP. Data from each EXP were analyzed using Proc MIXED, with and without PP as a covariant; significant differences ($P \leq 0.05$) were separated using Tukey HSD. For all EXP, PP influenced each processing variable ($P < 0.05$) except for EXP 3, whereas PP had no effect on drum weight (WT) and drum yield (YD; $P > 0.05$). In EXP 1, before utilizing PP, drum WT and YD, and leg WT were significantly affected by TRT ($P < 0.05$). Accounting for PP as a covariant maintained the significance for drum WT and YD and leg WT and led to the significance of wing WT and leg YD ($P < 0.05$). In EXP 2, none of the processing metrics were found to be significant due to TRT effect ($P > 0.05$). Utilizing PP as a covariant, reduced P-values obtained for breast, total breast, and wing (WT and YD), but did not result in significant TRT differences. In EXP 3, breast WT and YD, total breast WT, drum YD, and leg YD were found to be significantly affected by TRT ($P < 0.05$). When PP was applied as a covariant, P-values for these variables decreased further, with the exception of leg YD, whereas the TRT effect was reduced ($P > 0.05$). Notably, use of PP also resulted in a TRT effect for total breast YD ($P < 0.05$). These data demonstrate that use of PP as a covariant can help reduce variation, especially in parts that are highly variable due to cutting technique (i.e., breast, wings), thereby more accurately representing the TRT effect on processing variables.

Key Words: processing; yield; statistical analysis; nutrition research; covariant

Student Competition: Immunology, Health and Disease II

41 The effects of possible alternative treatments against *Ascaridia galli* on nutrient digestibility in infected laying hens. Maria Tereza Bethonico Terra^{1*}, Joseph Gulizia¹, Patricia Carvalho¹, Leopoldo Almeida², Jose Hernandez¹, Sureerat Thuekeaw³, Brigid McCrea¹, Wilmer Pacheco¹, Ruediger Hauck¹, ¹*Auburn University, Auburn, Alabama, United States*, ²*UFPR, Curitiba, Paraná, Brazil*, ³*Chulalongkorn University, Pathumwan District, Bangkok, Thailand*.

Prevalence of endoparasites is higher in alternative poultry rearing systems than conventional, especially of nematodes such as *Ascaridia galli*. Infections with *A. galli* can depress egg production in laying hens, possible mechanisms are negative influences on intestinal microbiota, increased energy use by the immune system, competition with the host for nutrients, or decreased nutrient digestibility. Natural products could be an alternative to treat nematodes. Pumpkin seeds and artemisia have shown efficacy against worms in other types of hosts. The aim of this study was to evaluate the influence of an *A. galli* infection on nutrient digestibility and to test if these treatments alleviated negative influences caused from the infection. A total of 72 Day-old layer-type birds were raised on the floor until 12 weeks of age when they were moved to cages. At 16 weeks, birds were divided into 3 groups: untreated control, treated with pumpkin seeds (1%) or *Artemisia absinthium* (0.02%). At 25 weeks, half of the birds were challenged with *A. galli*, resulting in six subgroups. Fecal exams were conducted weekly to check for worm eggs. At 34 weeks, titanium dioxide was added (0.5%) to the feed. At 35 weeks, birds were euthanized, and the intestines were checked for worms. Pools of ileal digesta (n = 4/subgroup) were collected for evaluation of digestibility of crude protein (CP) (%), calcium (%), phosphorus (%) and apparent ileal digestible energy (AIDE, kcal/kg). Data was tested by a two-way ANOVA. Means were compared using Tukey HSD. Differences were considered significant if $p \leq 0.05$. Birds started shedding worm eggs on week 4 and 5 post-infection (P.I.). There were numerical differences between groups and birds treated with artemisia had the highest average number of eggs per gram with 596 eggs per gram (epg), followed by pumpkin seed-treated birds with 521 epg and the controls with 364 epg. There were no differences between groups for the average worm count. On the other hand, for all nutrients, the apparent ileal digestibility was significantly lower in birds challenged with *A. galli* when compared to the unchallenged birds. An interaction effect between challenge status and different treatments was also observed for the digestibility of CP, calcium, and AIDE. Regarding the different treatments, challenged birds fed with the control had lower calcium digestibility than birds fed with either pumpkin seeds or artemisia ($p \leq 0.05$). Our findings suggest that *A. galli* can reduce the digestibility of nutrients and the use of pumpkin

seeds and artemisia might contribute to reduction of negative effects even though an impact on worm egg shedding and worm count was not observed.

Key Words: Digestibility; Laying hens; Roundworms; *Artemisia absinthium*; Pumpkin seeds

42 Antimicrobial and immunomodulatory effects of tannic acid supplementation in broilers infected with *Salmonella Typhimurium*. Janghan Choi^{1*}, Brett Marshall¹, Hanseo Ko¹, Hanyi Shi¹, Amit Singh¹, Harshavardhan Thippareddi¹, Steven Hollday¹, Robert Gogal¹, Woo Kim¹, ¹*University of Georgia, Athens, Georgia*.

Infection by *Salmonella Typhimurium*, a food-borne pathogen, can reduce the poultry production efficiency. The objective of this study was to investigate the effects of tannic acid (TA) supplementation on growth performance, *Salmonella* colonization, gut barrier integrity, serum endotoxin levels, antioxidant capacity, gut health, and immune function in broilers infected with the *Salmonella enterica* serovar Typhimurium nalidixic acid resistant strain (ST^{NR}). A total of 546 one-day-old broilers were arbitrarily allocated into 6 treatments including 1) Sham-challenged control (SCC; birds fed a basal diet and administered peptone water); 2) Challenged control (CC; birds fed a basal diet and inoculated with 10⁸ ST^{NR}); 3) Tannic acid 0.25 (TA0.25; CC + 0.25 g/kg TA); 4) TA0.5 (CC + 0.5 g/kg TA); 5) TA1 (CC + 1 g/kg TA); and 6) TA2 (CC + 2 g/kg TA). On D 7, supplemental TA linearly reduced ST^{NR} colonization in the ceca ($P < 0.01$). On D 7 to 21, average daily gain tended to be linearly increased due to supplemental TA ($P = 0.1$). The serum endotoxin levels were quadratically decreased due to supplemental TA on D 21 ($P < 0.05$). Supplemental TA quadratically increased ileal villus height (VH; $P < 0.05$), and the TA0.25 group had higher ileal VH compared to the CC group ($P < 0.05$). Supplemental TA linearly increased percentage of peripheral blood CD8⁺ T cells on D 18 ($P < 0.01$). The TA0.5 group had significantly lower lymphocyte numbers compared to the CC groups ($P < 0.05$). The abundance of monocytes linearly increased with TA supplementation ($P < 0.01$). Broilers supplemented with TA had reduced ST^{NR} colonization, increased growth performance, decreased serum endotoxin levels, enhanced gut health in the broilers, and stimulated the immune system in broilers infected with ST^{NR}.

Key Words: broilers; immunity; *Salmonella Typhimurium*; Tannic acid; Antimicrobials

43 Effects of synbiotic supplementation as an antibiotic growth promoter replacement on cecal *Campylobacter jejuni* load in broilers challenged with *C. jejuni*. Emily Cason^{1*}, Walid Al Hakeem¹, Daniel Adams¹, Revathi Shanmugasundaram², Ramesh Selvaraj¹,

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Synbiotic supplementation alters the gut microbiota to increase beneficial bacteria and decrease pathogenic bacteria. Synbiotics can improve production performance, decrease cecal *Campylobacter jejuni* load and colonization, and improve modifying immunological responses and can be used as an alternative to antibiotic growth promoters in poultry production. This study aimed to determine the effects of synbiotic supplementation as an antibiotic replacement in broilers challenged with *C. jejuni*. A total of 336 day old broiler chicks were distributed into four treatment groups: Control, Synbiotic, Antibiotic + Challenge, and Synbiotic + Challenge. There were six replicate pens per treatment, with 14 birds per pen. At 21 days of age, birds were orally inoculated with PBS or 1×10^8 CFU/bird of *C. jejuni*. Antibiotics (Virginiamycin) and synbiotics (Poultristar, DSM) were supplemented in feed at 20 mg/kg and 20 g/1000 birds/day respectively. Feed intake and body weight were measured weekly. On 3, 7, and 14 dpi, gut permeability was measured by FITC-dextran assay, *C. jejuni* loads in cecal content, ileal tissue, and livers were quantified by plating, splenocyte nitric oxide production was quantified using Ricca reagent, and cecal tonsil CD4⁺:CD8⁺ and CD4⁺:CD25⁺ percentage were measured by flow cytometry. Data were statistically analyzed using JMP Pro 15, by One Way ANOVA, followed by Tukey's test, non-parametric Kruskal-Wallis test, and by Dunn's test, when appropriate. There were no significant differences ($P > 0.05$) in BWG, FCR, or gut permeability between the treatment groups at the time points studied between any of the treatment groups. At 7 and 14 dpi, birds in the Synbiotic + Challenge group had 0.70 and 0.67 log₁₀ decrease ($P < 0.05$) in cecal *C. jejuni* load compared to the Antibiotic + Challenge groups. At 7 dpi, cecal tonsil CD4⁺:CD8⁺ was significantly higher ($P < 0.05$) in the Synbiotic + Challenge and Antibiotic + Challenge groups by 0.19 and 0.29 points than in the Synbiotic and Control groups, respectively. At 3 dpi, cecal tonsil CD4⁺:CD25⁺ was significantly higher ($P < 0.05$) in the Synbiotic + Challenge than in the Synbiotic group by 7.16 points. It can be concluded that synbiotic supplementation decreases *C. jejuni* loads in broilers compared to antibiotic supplementation and synbiotics can be applied to replace antibiotics during *C. jejuni* infections of poultry.

Key Words: Campylobacter jejuni; Broiler; Antibiotic; Immunology; Synbiotic

44 Effect of different doses of *Salmonella* Reading on mRNA abundance of immune response related genes in turkey poults. Davis Fenster^{1*}, Candice Blue¹, Laney Froebel¹, Rami Dalloul¹, ¹University of Georgia, Athens, Georgia, United States.

Recent outbreaks of salmonellosis linked to poultry meat and meat products are of serious concern regarding the potential risk of foodborne illnesses. *Salmonella* Reading

has recently emerged in turkey production as a major cause of foodborne outbreaks associated with turkey meat and meat products. The main objective of this study was to determine the effect of different doses of *S. Reading* on mRNA abundance of immune response related genes in turkey poults. Day (d)-old male Nicholas poults (n=487) were weighed and separated into four groups (n=112/group) based on the *S. Reading* inoculation dose: (1) 1×10^2 , (2) 1×10^4 , (3) 1×10^6 , or (4) 1×10^8 . Additional poults (n=39) were assigned to a control group and did not receive *S. Reading*. Each treatment group had four replicate pens (n=28/pen). On d 7, poults in the treatment groups were orally gavaged with novobiocin- and nalidixic acid-resistant *S. Reading*. Poults in the control group were orally gavaged with sterile PBS. On d 9 and d 21, spleen and cecal tonsil samples were collected from four birds per treatment to measure mRNA abundance of interleukin (IL)-1 β , IL-10, interferon (IFN)- γ , inducible nitric oxide synthase (iNOS), and Toll-like receptors (TLR)-2A, TLR-2B, TLR-4, TLR-5, and TLR-15. Data were analyzed using ANOVA procedure of JMP (Pro 16) and significance ($P \leq 0.05$) between groups were determined by LSD test. On d 9, group 4 (highest dose) had significantly greater mRNA abundance of TLR-2A, TLR-5, TLR-15, IL-10, and IFN- γ compared to all other groups, and significantly greater mRNA abundance of TLR-4 compared to the control and group 1 in the cecal tonsils ($P < 0.05$). Additionally, groups 2, 3, and 4 had significantly greater mRNA abundance of TLR-2B, IL-1 β , and iNOS in the cecal tonsils compared to the control group. No significant differences ($P > 0.05$) in mRNA abundance for any of the genes measured were observed in the cecal tonsils on d 21 or in the spleen on d 9 and d 21. However, mRNA abundance of TLR-5, TLR-15, IFN- γ , and iNOS was numerically greater for group 1 (lowest dose) in the spleen on d 21. Overall, increasing doses of *S. Reading* differentially affected the mRNA abundance of immune response genes in the cecal tonsils 2 days post inoculation. These data indicate that higher doses of *S. Reading* induced a stronger initial immune response in turkey poults. The present findings may be useful in developing targeted products that enhance specific host immune responses to mitigate *S. Reading* prevalence at the flock level, and ultimately reduce the incidence of foodborne outbreaks associated with turkey meat products.

Key Words: dose; mRNA; turkey; immunity; Salmonella Reading

45 Local mRNA expression of cytokines during the first seven days following the intradermal administration of autogenous *Salmonella* vaccines in previously vaccinated Light-brown Leghorn pullets. Chrysta Beck^{1*}, Jossie Santamaria¹, Marites Sales¹, Gisela Erf¹, ¹University of Arkansas System, Division of Agriculture, Fayetteville, Arkansas, United States.

Salmonella vaccines are administered to lower chick morbidity and prevent human salmonellosis from poultry

products. Few studies have investigated cellular responses to *Salmonella* vaccines at the site of administration. Using the growing feather (GF) pulp *in vivo* test-system, the objective was to analyze the mRNA expression of various cytokines in the GF pulp (a skin derivative) after an intradermal (i.d.) secondary injection with *Salmonella* vaccines or vaccine components. Light-brown Leghorn pullets (14-wk-old) were subcutaneously injected with autogenous *Salmonella* vaccines (SV1 or SV2, proprietary formulations), *S. Enteritidis* lipopolysaccharide, or vaccine vehicle for the primary response. Four weeks later, GF pulps were i.d. injected with the respective primary treatment to examine local immune activities in response to the secondary administrations (n=4 pullets/treatment). Injected GFs were collected before (0 d) and at 6 h, 1 d, 2 d, 3 d, 5 d, and 7 d post-GF injection (21 GF/bird) to monitor leukocyte infiltration profiles and quantify cytokine mRNA expression (IL-1 β , IL-6, IFN- γ , IL-12 α , TNF- α , IL-8, IL-4, IL-13, IL-17 α , IL-10, TGF- β 1) by qRT-PCR. Data were analyzed by two-way ANOVA (time vs. treatment) with significance established at $P \leq 0.05$. Secondary i.d. injection with SV1 or SV2 initiated leukocyte infiltration profiles dominated by heterophils followed by macrophages and CD4+ T cells ($P < 0.05$). Cytokine mRNA expression revealed that the secondary i.d. injection with SV1 or SV2 stimulated inflammatory responses with IL-1 β , IL-6, IL-12 α , and IL-8 expression reaching maximal levels by 2 d post-GF injection ($P \leq 0.05$). There were marginally significant ($P = 0.10$) increases in anti-inflammatory cytokines expression (IL-10 and TGF- β 1) over time. IFN- γ expression (the signature Th1 cytokine) was not influenced by treatment or time ($P > 0.05$). IL-13 expression (a Th2 cytokine) was elevated in SV1-injected GFs by 2 d-post injection, whereas IL-17 α expression (a Th17 cytokine) was elevated ($P < 0.05$) with both SV1 and SV2 at 6 h-, 2 d-, and 3 d-post injection. In summary, local immune activities initiated by a secondary i.d. *Salmonella* administration included mRNA expression of pro-inflammatory cytokines accompanied by increased expression of Th17- and Th2-, but not Th1-cytokines. New knowledge gained from studies that investigate cellular responses to killed- as well as live-*Salmonella* vaccines may find applications in the development and optimization of effective *Salmonella* vaccines in poultry production.

Key Words: Cytokine; *Salmonella*; Inflammation; Vaccine; Leukocyte

46 Immunological profile of broiler chicks infected with *Salmonella* Typhimurium. Allison Milby^{1*}, Yuhua Farnell¹, Dan Zhao¹, Luc Berghman¹, Craig Lanio², Melissa Muller², Morgan Farnell¹, ¹Texas A&M University, College Station, Texas, United States, ²Millipore Sigma, Saint Louis, Missouri, United States.

Salmonellosis is a zoonotic disease caused by *Salmonella enterica* serovars resulting in an estimated 1.4 million cases of foodborne illness and 400 deaths in the United States

annually. Poultry infected with *Salmonella* Typhimurium (ST) are asymptomatic but can continually shed into the environment causing cross contamination of carcasses. The avian immune system responds to *Salmonella* colonization by expression of cytokines, chemokines, and other soluble factors. We hypothesized that the immune status of ST challenged chicks would differ from uninfected neonatal broilers. The objective of this experiment was to evaluate 12 analytes by a commercially available multiplex ELISA in ST challenged broilers. Day of hatch male broiler chicks were randomly allocated into two treatment groups (control and ST). After three days of acclimation, a ½ mL oral gavage of sterile diluent or 10⁹ cfu/mL of ST was given to each chick. All chicks were euthanized 24 h post infection. Blood was obtained immediately after death by cardiac puncture, and serum separated for later analysis (n = 30 chicks per treatment). Data were analyzed via a Student's *t*-test with significant differences found at $p \leq 0.05$. Increased expression of **pro-inflammatory cytokines**- interleukin-6 (IL-6; $p = 0.0025$), IL-16 ($p = 0.0196$), and IL-21 ($p = 0.0066$); **anti-inflammatory cytokines**- IL-10 ($p = 0.0047$); **chemokines**- regulated on activation, normal T-cell expressed and secreted (RANTES/CCL5; $p = 0.0002$), macrophage inflammatory protein-1 β (MIP-1 β ; $p = 0.0019$), and MIP-3 α ($p = 0.0050$); **colony stimulating factors**- macrophage colony-stimulating factor (M-CSF; $p = 0.0239$); and **growth factors**- vascular endothelial growth factor (VEGF; $p = 0.0022$) were observed in the serum of the ST group when compared to the control. No significant differences were observed in IL-2 ($p = 0.1778$), IFN γ ($p = 0.1316$), and IFN α ($p = 0.5044$). These data suggest that an innate mucosal immune response to ST infection in broiler chicks was detected. The increased pro-inflammatory cytokines, chemokines, and colony stimulating factors correspond to inflammatory processes that have been previously reported with similar studies. However, upregulation of the anti-inflammatory cytokine IL-10 was unexpected and seems to contradict the upregulation of other cytokines observed. We find the increase in VEGF compelling, as this indicates potential tissue repair and angiogenesis in birds infected with ST. These data may continue to improve our understanding of ST colonization in broiler chickens.

Key Words: cytokine; immune response; *Salmonella* Typhimurium; poultry; multiplex immunoassay

47 Bird age did not significantly influence glycolytic metabolic response of peripheral blood mononuclear cells isolated from LPS-challenged Leghorn roosters. Kayla Elmore^{1*}, Elizabeth Bobeck¹, ¹Iowa State University, Ames, Iowa, United States.

Endotoxins including lipopolysaccharide (LPS) induce inflammatory responses in poultry. Prior work using cultured macrophages has reported that LPS tolerance may reduce glycolytic respiration, but *in vivo* studies are lacking. The objectives were to compare baseline and stressed

metabolic phenotypes of isolated peripheral blood mononuclear cells (PBMCs) from leghorn strains and identify the fuel preference of cells under stressed conditions. Four treatments were used under a 2x2 factorial arrangement with 2 age groups (1 yr or 2.5+ yrs) and 2 challenges (saline or LPS). A total of 20 white leghorn roosters were randomly assigned treatments and were inoculated intramuscularly across 4 sites with either saline (CON) or LPS (*Escherichia coli* O55:B5, LPS) at 1 mg/kg BW on d1. Blood was collected at baseline before inoculation, 6 hr post-inoculation (pi), and 24 hrpi. Cloacal temperature was recorded at each time point, with bodyweight recorded at baseline and 24 hrpi. Peripheral blood mononuclear cells (PBMC) were isolated from whole blood and Seahorse metabolic analysis was conducted using Real-Time ATP Rate Assay and Glycolytic Rate Assay kits (Agilent). The Real-Time ATP Rate Assay measures the rate of adenosine triphosphate (ATP) production from mitochondrial oxidative phosphorylation and glycolysis, while the Glycolytic Rate Assay measures glycolysis through proton efflux rate quantification. Assays report oxygen consumption rate (OCR) as a measure of glycolysis and extracellular acidification rate (ECAR) measuring mitochondrial respiration. Statistical analysis was performed using the MIXED procedure with fixed effects of age followed by Tukey-Kramer adjustment (SAS 9.4, significance denoted at $P \leq 0.05$). Younger birds weighed 0.523 kg more ($P < 0.0001$) before inoculation and had increased body temperatures at 6hrpi when compared to the older group ($P = 0.0285$ and $P = 0.0024$, respectively). No bodyweight or temperature differences were observed at 24 hrpi compared to initial baseline weights. Despite evidence of a hyperthermic response in the younger group, the metabolic results show that the effect of treatment ($P = 0.8193$) nor age ($P = 0.1135$) did not significantly influence cellular metabolic preference. Timepoint (baseline, 6, or 24hrpi) had a significant effect on response ($P < 0.01$), as did challenge within the assay ($P < 0.01$), showing the PBMC were able to respond to a challenge within the metabolic assays. These results suggest that adult birds with previous exposure to chronic environmental LPS may respond similarly to an acute LPS challenge despite age differences.

Key Words: immunometabolism; roosters; LPS; Seahorse metabolic assay

48 Dietary resistant starch prevents post-antibiotic intestinal impairment by reducing gut dysbiosis and preventing intestinal inflammation in meat ducks. Simeng Qin^{1*}, Keying Zhang¹, Xuemei Ding¹, Jianping Wang¹, Shiping Bai¹, Qiufeng Zeng¹, ¹*Sichuan Agricultural University, Chengdu, Sichuan, China.*

Antibiotic treatment, commonly prescribed for bacterial infections, depletes and subsequently causes long-term alterations in intestinal microbiota composition. Knowing the importance of the microbiome in the regulation of intestinal barrier, in this study thus was conducted to

investigate the effect of post-antibiotic treatment on gut health and the protective effect of resistant starch (RS), which has been indicated that RS could improve gut microbiota and alleviate inflammation as a functional ingredient. 7-d-old ducks (367.3 ± 10.38 g) were allocated to control or antibiotic groups, which was treated with broad-spectrum antibiotics (ampicillin 1.0 g/L and neomycin 0.8 g/L) via sterile drinking water. After 7 d, antibiotic treatment was stopped, and the ducks were treated for 7 d with the basic diet alone or containing 12% raw potato starch (RPS) as the resource of RS. At 21 d of age, one bird per cage was selected and sampled. One-way analysis of variance followed by Tukey's *post hoc* test was conducted to examine statistical significance. Compared to control group, the antibiotic treatment did not affect body weight, ileal length and weight of ducks, whereas it significantly increased the concentrations of interleukin (IL)-1 β and interferon (IFN)- γ ($P = 0.071$) in serum, as well as the gene expression of tumor necrosis factor alpha (TNF- α), IL-1 β ($P = 0.068$) and IL-6 in ileum. Intestinal microbiome repopulation at 1-weeks post-antibiotic treatment resulted in the decrease in the alpha and beta diversity, along with decreased Firmicutes: Bacteroidetes ratio and increased the abundance of Proteobacteria (all $P < 0.05$). Supplementation with 12% RPS prevented the post antibiotic-induced inflammatory response by suppressing the concentration of TNF- α and IL-1 β in serum, as well as the gene expression of TNF- α , IL-1 β ($P = 0.061$) and IL-6 in ileum ($P < 0.05$). Furthermore, dietary 12% RPS administration could increase the post-antibiotic elevation of the Firmicutes: Bacteroidetes ratio and prevent the decline in microbial diversity. At the genus levels, diets with 12% RPS induced higher relative abundances of *Sellimonas* and *Ruminococcaceae* UCG-014 ($P < 0.05$). These data underscore the importance of microbial dysbiosis in the regulation of intestinal health of meat ducks such as triggering inflammation, as well as identify 12% RPS as novel therapies for preventing these adverse effects.

Key Words: antibiotic; gut microbiota; Inflammation; meat ducks; Resistant starch

49 Phenotypic virulence characterization of avian pathogenic *Escherichia coli* isolates from broiler breeders with colibacillosis. Jiddu Joseph^{1*}, Christopher Magee², Linan Jia¹, Li Zhang¹, Pratima Adhikari¹, Reshma Ramachandran¹, ¹*Mississippi State University, Starkville, Mississippi, United States,* ²*Poultry Research Unit, Starkville, Mississippi, United States.*

Avian pathogenic *Escherichia coli* (APEC) is the organism responsible for colibacillosis, a disease which causes severe economic loss to the poultry industry worldwide. Vertical transmission of APEC from broiler breeders through contaminated eggs to offspring is a major route for its transmission and thus, necessitates its control in breeders. However, there is only limited information on the virulence characteristics of APEC isolates from broiler breeders.

Hence, the objective of this study was to assess the phenotypic virulence characteristics of 28 APEC isolates collected from broiler breeders with colibacillosis obtained from the Mississippi Poultry Research and Diagnostic Laboratory. Two hundred and fifty-six one-day-old Ross708 × YPM female broiler chicks were divided into 32 groups (n=8 chicks/group) for 28 isolates and 4 controls (2 sham-PBS injected and 2 non-injected groups). Each chick was subcutaneously injected in the neck region with 0.1 mL of an overnight culture of APEC isolate reconstituted in PBS to a final concentration of 1×10^8 CFU/mL. The chicks were monitored 4 times daily for 7 days post-challenge; mortality and postmortem lesions were recorded and liver, heart, and reproductive organs were collected from 4 chicks/group to identify *E. coli* colonization. To evaluate the virulence of APEC isolates and to classify them, a pathogenicity score (PS) was assigned to each isolate based on the day of chick death and lesions during necropsy. Further, Pearson correlation analysis was performed using SAS 9.4 to examine the relationship of PS with various virulence-associated genes. Results showed that 4 isolates (14.30%) caused 100% mortality and 8 isolates (28.60%) caused 0% mortality within 7 days post-challenge. Based on PS, among the 28 isolates, 11 (39.30%) were classified as highly pathogenic, 6 (21.40%) as intermediate and 11 (39.30%) as low pathogenic. Positive correlations were observed for PS with genes for iron acquisition, *iroN* ($R=0.50$, $P=0.01$) and *iutA* ($R=0.40$, $P=0.05$), and the gene for increased serum survival, *iss* ($R=0.50$, $P=0.01$). In conclusion, this study enabled us to differentiate the APEC strains isolated from broiler breeders with colibacillosis based on their virulence potential in day-old chicks and establish a relationship between pathogenicity and the presence of certain virulence-associated genes that are part of APEC minimal predictors. Our findings provide insight to further research on the virulence characteristics of APEC causing infections in broiler breeders to develop an APEC challenge model as well as an effective vaccine.

Key Words: avian pathogenic *Escherichia coli*; broiler breeder; colibacillosis; virulence characterization; pathogenicity score

50 Effects of *in ovo* injection of an *Escherichia coli* vaccine on the posthatch growth of commercial layer chicks. Lauren Lindsey^{1*}, Jeff Evans², Katie Elliott², Saman Fatemi¹, Ayoub Mousstaaid¹, Patrick Gerard³, David Peebles¹, ¹Mississippi State University, Starkville, Mississippi, United States, ²USDA-ARS, Starkville, Mississippi, United States, ³School of Mathematical and Statistical Sciences, Clemson, South Carolina, United States.

In the commercial layer industry, avian pathogenic *Escherichia coli* (APEC) infections can lead to significant financial loss. The Poulvac® *E. coli* vaccine (PECV; Zoetis, Parsippany, NJ) is currently the

recommended vaccine to combat APEC. The effect of *in ovo* administration of PECV on the hatchability of layer hatching eggs has been tested in a previous companion trial. The purpose of this experiment was to examine the effects of the *in ovo* administration of different doses of the PECV in fertile Hy-Line W-36 layer hatching eggs on chick posthatch growth and development. Vaccine treatments included non-injected and diluent-injected controls, and the injection of diluent containing a full dose, or 1×10^{-2} , 1×10^{-4} , 1×10^{-6} dilutions of the full dose of the vaccine. Injections (50 µL) targeted the amnion on day 18 of incubation. A full dose was calculated to contain 2.65×10^7 CFU of *E. coli* bacteria. Cumulative mortality through 3 wk posthatch was 5.41 % in the full dose treatment, with no mortalities observed in the other 5 treatments. On day of hatch, as well as at 1, 2, and 3 wk posthatch, mean chick BW per treatment was calculated. Chick length was calculated at 3 wk posthatch. Mean chick BW did not differ significantly ($P = 0.308$) between treatments at hatch, but differed significantly ($P < 0.0001$) between treatments at 1, 2, and 3 wk of age. At all 3 posthatch ages, chicks that had been injected with diluent alone had the highest mean chick BW, with chicks that had been injected with a full dose of vaccine having the lowest mean chick BW. By the third week, the chicks injected with a full dose of vaccine weighed nearly 20 g less than the diluent-injected chicks. Chick length was significantly ($P = 0.045$) different between treatments, with the body length of chicks in the full dose treatment being shorter than in the 2 control groups. Overall, layer chicken embryos were able to survive through 3 wk posthatch after having received the PECV by *in ovo* injection. However, the full dose of the PECV increased cumulative chick mortality, and chick BW decreased with an increase in PECV concentration through 3 wk posthatch.

Key Words: body weight; *in ovo*; layers; *E. coli*; posthatch

51 Influence of Dietary Ginger Root Extract on the Expression Levels of Antioxidant Defense Enzymes and Mucin Genes in the Intestine of Broiler Chicks. Olubunmi Apalowo^{1*}, Adedeji Adetunji¹, Md Maruf Khan¹, Yewande Fasina¹, ¹North Carolina A&T State University, Greensboro, North Carolina, United States.

The intestinal epithelium of poultry is continuously exposed to oxidative stressors associated with feed and environmental conditions. Ginger rhizome (*Zingiber officinale*; ginger root) contain bioactive compounds such as gingerols and gingerdiols that possess strong antioxidant activity. A total of 432 day-old chicks (Ross 708 strain) were obtained from a commercial hatchery and subjected to a 21-day experiment to investigate the effect of ginger root extract (GRE) on the expression of genes coding for antioxidant defense enzymes [Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPX)] and mucin (MUC2). Chicks were weighed and randomly assigned to six dietary treatments of varying ginger concentrations in a completely randomized design as

follows: Treatment **CON** (fed corn-soybean meal (**SBM**) basal), Treatment **MX** (fed basal diet containing bacitracin methylene disalicylate (**BMD**) antibiotic at 0.055 g/kg diet), Treatment **GRE1** (fed basal diet containing 0.37% GRE), Treatment **GRE2** (fed basal diet containing 0.75% GRE), Treatment **GRE3** (fed basal diet containing 1.5% GRE), and Treatment **GRE4** (fed basal diet containing 3% GRE). Each treatment consisted of 6 replicate pens, with each pen housing 12 chicks. Body weight (**BW**), body weight gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**) was monitored throughout the experiment. On d 6 and 13 of experiment, intestinal ileal tissue segments were collected and subjected to RNA isolation procedures. Resulting RNA samples were reverse transcribed to cDNA that were subjected to Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) to determine the expression levels of SOD, CAT, GPX, and mucin (MUC2) genes. Results showed that with the exception of GRE4 treatment, growth performance parameters were similar ($P > 0.05$) for all treatments. Ileal gene expression results on d 6 showed that SOD was upregulated ($P < 0.05$) in GRE3 chicks (2.43 folds) compared to CON and MX chicks. The CAT gene was downregulated in MX and GRE treatments ($P < 0.05$; 0.32 to 0.71) compared to CON, while no difference ($P < 0.05$) was observed in the expression of GPX gene among treatments. Expression of MUC2 gene in the ileum of GRE2 (1.83 fold) and GRE3 (1.93 fold) was upregulated ($P < 0.05$) compared to CON and MX. It was concluded that GRE may mitigate intestinal oxidative stress through upregulation of SOD enzyme (antioxidant defense) and MUC2 genes.

Key Words: Mucin; Ginger root extract; Intestinal oxidative stress; Superoxide dismutase; Broiler Chicks

52 Case report: high and rapid mortality associated with anaerobic bacterial infections in a turkey research trial. Chloe O'Brien^{1*}, Catherine Fudge¹, Rocio Crespo¹, Chongxiao Chen¹, Zohreh Mehdipour¹, Raveendra Kulkarni¹, ¹North Carolina State University, Raleigh, North Carolina, United States.

This is a case report pertaining to unanticipated high and rapid mortality in a turkey research trial, which might be associated with *Clostridium perfringens* and *E.coli* infection or their toxins. In case 1, day-old turkey poults were placed in 36 battery cages with 10 birds/cage. At week 2, birds were cloacally inoculated with 1 ml of *Histomonas meleagridis* culture and were held upside down for two minutes to ensure successful infection. Within 48 h post-inoculation, the mortality reached 16.7%, and in the following 48 h, the mortality dropped dramatically to 1.4%. 6 birds were sent to Rollins Animal Disease Lab (Raleigh, NC) for inspection, where results showed marked gizzard koilin layer necrosis with a heavy growth of *C. perfringens*. Liver surface swaps were positive for *E.coli* and *Streptococcus gallolyticus*. Following case 1, we did a complete clean out and disinfection to ensure there

was no contamination for the next trial (case 2). However, similar issues were observed. In case 2, 48 h post-inoculation, mortality reached 51% and dropped to 2% for the next 48h. Between these two cases, it is noticed that only the birds being handled and inoculated were experiencing accelerated mortality. We hypothesized it might be caused by handling stress, inoculation stress, toxins in the media, or potential contamination in the *H. meleagridis* cultures. To determine the factors in rapid mortality, two pilot trials were conducted. In trial 1: 50 poults were assigned to 5 cages with the treatments: 1. handling only (upside down for 2 mins), 2. handling + 1 mL water (cloacal inoculation), 3. handling + 1 mL media (no parasites or bacteria), 4. handling + 1 mL *H. meleagridis* parasite culture, and 5. 1 mL *H. meleagridis* parasite culture. Within 48h, only treatments 4 and 5 had high mortality with 50% in both. To further explore if the parasite culture was the issue, in trial 2, we inoculated birds with different amounts of culture (10 birds/level). Mortality results showed 70% in 1.5mL and 40% in 1ml and 0% in 0.25ml inoculation. Cultures were also sent for *C. perfringens* identification, which showed that the cultures used in the infection were positive. Further genotyping, microbiome, and toxins analysis in the cultures are under process. In summary, the cultures seem to play a critical role in these cases. The *H. meleagridis* were cultured anaerobically with bacteria and rice powder as energy source, which may favor *C. perfringens* growth and toxin production and accumulation. In the past, such a rapid mortality has rarely been seen. Other co-factors, such as stresses, other bacteria, or potential colon damage during inoculation, may have contributed to the rapid and high mortality.

Key Words: Clostridium perfringens; E. coli; Histomonas meleagridis; Co-infection; Cloacal inoculation

53 Detection of antimicrobial resistance genes in Lactobacillus spp. from poultry probiotic products and their horizontal transfer among Escherichia coli. Md. Rokon-Uz-Zaman^{1*}, Anica Bushra¹, Tanjida Akter Pospo¹, Monika Akter Runa¹, Sadia Tasnuva¹, Mst. Sonia Parvin¹, Md. Taohidul Islam¹, ¹Bangladesh Agricultural University, Mymensingh, Bangladesh.

Nowadays, the use of probiotics has been augmented in poultry production. However, they should be free from any transferable resistance genes. The study was conducted to identify the antimicrobial resistance genes (ARGs) in *Lactobacillus* spp. from commercially available poultry probiotic products and their potential to spread among *Escherichia coli*. A total of 35 probiotic products from seven brands/companies (five from each brand) commonly used in poultry were purchased from veterinary pharmacies. *Lactobacillus* spp. were isolated and identified based on cultural, biochemical, and molecular findings. All the isolates (n = 35) were screened for the presence of some ARGs such as β -lactamase encoding genes (*bla*TEM, *bla*CTXM-1, and *bla*CTXM-2), plasmid-

mediated quinolone resistance genes (*qnrA*, *qnrB*, and *qnrS*), and tetracycline resistance genes (*tetA* and *tetB*). The co-culture of *Lactobacillus* spp. and *E. coli* was performed to evaluate the transfer of resistance genes. The antimicrobial susceptibility test (AST) was also performed by the disc diffusion method. Five *Lactobacillus* spp. isolates from three brands were positive for one or more ARGs. The *qnrS* was detected in four isolates. *bla*TEM and *tetB* were detected in two isolates. One isolate contained *bla*CTX-M-1, *bla*CTX-M-2, and *tetA* genes. Brand-wise analysis revealed that one isolate from *Brand 4* was positive for *bla*TEM, *bla*CTX-M-1, *bla*CTX-M-2, *qnrS*, and *tetA* genes; one isolate from *Brand 2* was positive for the *bla*TEM gene; and three isolates from *Brand 7* harbored the *qnrS* gene. The co-culture

resulted in the transmission of *qnrS*, CTX-M-1, and *tetA* from *Lactobacillus* spp. to *E. coli*. Results of AST revealed that the highest resistance was observed to cefepime and cefotaxime, followed by penicillin G, oxacillin, cefuroxime, and ofloxacin. The least resistance was observed against ciprofloxacin, nalidixic acid, vancomycin, and clindamycin. The results of the present study indicate the potential risk of horizontal spread of antimicrobial resistance through probiotic bacteria among the poultry population. Therefore, it is very necessary to check ARGs along with other attributes of probiotic bacteria to avoid the inclusion of resistant strains in probiotics.

Key Words: Probiotics; *Lactobacillus*; Poultry; Antimicrobial resistance gene; Horizontal transfer

Student Competition: Genetics and Molecular Biology II

54 Epigenetic Regulation of Host Defense Peptide Synthesis: Synergy between Histone Deacetylase Inhibitors and DNA/Histone Methyltransferase Inhibitors. Melanie Whitmore^{1*}, Glenn Zhang¹, ¹*Oklahoma State University, Stillwater, Oklahoma, United States.*

Host defense peptides (HDPs) are an integral part of the innate immune system as the first line of defense against infections. Modulation of HDP synthesis has emerged as a promising host-directed approach to fight against infections. Inhibition of histone deacetylation or DNA methylation has been found to enhance HDP gene expression. In this study, we explored a possible synergy in HDP gene induction between histone deacetylase inhibitors (HDACi) and DNA/histone methyltransferase inhibitors (DNMTi/HMTi). Two chicken macrophage cell lines were treated with structurally distinct HDACi, HMTi, or DNMTi individually or in combination for 24 h, followed by total RNA extraction and RT-qPCR analysis of HDP gene expression. Statistical analysis was performed using ANOVA then Tukey's multiple comparisons test. Each epigenetic compound was found to be capable of inducing HDP expression ($P < 0.05$). To our surprise, a combination of HDACi and HMTi or HDACi and DNMTi showed a strong synergy to induce the expressions of most HDP genes. The HDP-inducing synergy between butyrate, an HDACi, and BIX01294, an HMTi, were further verified in chicken peripheral blood mononuclear cells. Furthermore, tight junction proteins such as claudin 1 were also synergistically induced by HDACi and HMTi. Overall, we conclude that HDP genes are regulated by epigenetic modifications. Strategies to increase histone acetylation while reducing DNA or histone methylation exert a synergistic effect on HDP induction and, therefore, have potential for the control and prevention of infectious diseases.

Key Words: host defense peptides; histone deacetylase inhibitors; histone methyltransferase inhibitors; DNA methyltransferase inhibitors; epigenetic modification

55 Heritability Estimates of Egg Quality Traits in Pekin Ducks and Their Relationship with Other Production Efficiency Traits. Carl Kroger^{1*}, Luiz Brito¹, Gregory Fraley², Bill Lee³, Darrin Karcher¹, ¹*Purdue University, Westport, Indiana, United States*, ²*Purdue University, West Lafayette, Indiana, United States*, ³*Maple Leaf Farms, Manchester, Indiana, United States.*

Pekin duck (*Anas platyrhynchos domesticus*) is the most widely consumed duck protein with nearly 35 million ducks produced annually in the USA and exported worldwide. Ducks are used not only for their meat production but also feathers that are commonly found in winter jackets and comforters. The duck meat market is expected to increase by 3% from 2021 to 2025. Therefore, genetically improving duck populations together with the implementation of more efficient nutritional and management strategies is

paramount for the long-term sustainability of the US duck industry. In this context, the main objectives of this study were to estimate heritability and variance components for various egg quality traits on Pekin ducks and determine their relationship to production efficiency traits. Egg quality traits of 305 Pekin hens were measured through three time points during their laying cycle [30, 32, and 35 weeks of age (woa)]. The hen's juvenile feed conversion ratio (FCR) and body weights at two different time points (2 and 5 woa) were measured to explore the relationship to the quality of eggs produced. The average and standard deviations for each egg quality trait are: egg volume (EV), 75.15 ± 6.01 ml; egg weight (EW), 82.74 ± 5.92 g; shell thickness (ST), 0.41 ± 0.02 mm; yolk weight (YW), 24.95 ± 2.32 g; shell strength (SS), $4,823.18 \pm 850.76$ N; and, vitelline membrane strength (VMS), 172.13 ± 67.99 N. A linear modeling approach was implemented to evaluate the influence of different factors on hen's efficiency traits and eggs produced. We found that FCR and age at lay influenced YW (0.0347, 2.73E-06; p-value at 32 woa, p-value at 35 woa). Five week BW and age at lay had an association with YW (p-value at 32 woa, p-value at 35 woa; 0.0163, 2.19e-05), EW (0.0044, 6.99e-08), ST (non-significant, 0.00171), and SS (0.0163, 2.19e-05). Variance components and heritability estimates were calculated for all the traits using the BLUPF90 software, the Restricted Maximum Likelihood (REML) method, and 9,418 individuals in the pedigree. The heritability estimates were 0.357 ± 0.076 (EV), 0.594 ± 0.057 (EW), 0.722 ± 0.04 (ST), 0.371 ± 0.076 (YW), 0.167 ± 0.079 (SS), and 0.186 ± 0.075 (VMS). These findings indicate that all the egg quality traits evaluated are moderately to highly heritable in Pekin Ducks and can be improved through direct genetic or genomic selection.

Key Words: Egg Quality; Poultry; Genetics; Genetic Parameter; Feed Conversion Ratio

56 Primary Growth Plate Chondrocyte Isolation, Culture, and Characterization from the Modern Broiler. Alison Ramser^{1*}, Elizabeth Greene¹, Narayan Rath², Sami Dridi¹, ¹*University of Arkansas, Fayetteville, Arkansas, United States*, ²*USDA/ARS, Fayetteville, Arkansas, United States.*

Poultry has made major improvements in the area of performance and efficiency. In order to maintain this upward trajectory, the modern broiler must be robust against disease, infection, and injury. Therefore, the study of common diseases in poultry needs relevant and appropriate models. Bacterial chondronecrosis with osteomyelitis (BCO) is a leading cause of lameness affecting the growth plates of the long bones. A major limitation in studying BCO is a lack of chicken immortalized chondrocyte cell. Isolation of primary chondrocyte cells is a means of studying diseases of the growth plate *in vitro*, but requires validation and characterization, including well-defined

protocols, to be considered a physiologically relevant model. In this study, isolation and high-density culturing of primary chondrocytes from 10-day old chicks (1 leg rendered approximately 1×10^6 cells) was followed by confirmation of cell type, identification of optimal phenotypic expression, and evaluation of cells functionality. Cell lysate and media were collected at each time point for analysis ($n = 3$). Relative expression of target genes was determined using real-time qPCR and protein was visualized and quantified via Western Blot and Alpha View software. Data were analyzed by one-way ANOVA using GraphPad. Live cell imaging was conducted using Cytation3 plate reader. This study is the result of 1 experiment with 3 technical replicates per measurement and based on previous pilot studies. Results showed a shift from COLII dominant expression in early-culture to COLI dominant expression by late-culture in conjunction with a loss of other chondrocyte markers such as Sox9, ACAN,

and COLXA1 ($P < 0.05$). Additionally, morphological changes seen through live cell imaging coincide with the shift of phenotype in mid- to late-culture periods indicating a dedifferentiated phenotype. The functionality of the cultured cells was confirmed using Brefeldin-A treatment which significantly reduced secretion of COLII by day 7 (d7) ($P < 0.05$). By evaluating mRNA abundances, protein expression and secretion of chondrocyte-specific factors on day d3, d7, d11, d14, d18, and d21 in culture, it was shown that avian growth plate chondrocytes under these conditions exhibit optimal phenotype between d3 to d7. These results provide an accurate road map to isolation and utilization of avian primary chondrocyte cells in the study of bone-related infections and diseases as well as the foundation for future work immortalizing or passaging.

Key Words: broiler; primary chondrocyte; growth plate

Student Competition: Physiology and Reproduction

57 Influence of light intensity, pre-harvest fasting, and storage time on total protein, uric acid, and creatinine in serum and plasma of broilers. Clauber Polese^{1*2}, Lucas Wachholz¹, Nilton Rohloff Junior¹, Cleison De Souza¹, Guilherme Silva Tesser¹, Lidiane Datch¹, Cristine Kaufmann¹, Cinthia Eyng¹, Charles Starkey², Ricardo Nunes¹, ¹Western Paraná State University, Marechal Cândido Rondon, Paraná, Brazil, ²Auburn University, Auburn, Alabama, United States.

Analysis of blood metabolites is routinely included in broiler research studies. However, there is little information available related to the impact of rearing conditions, pre-blood collection fasting times, which fraction is optimal for which metabolites, or how long they can be stored. Therefore, the objective of this completely randomized design experiment with a $2 \times 7 \times 5 \times 2$ factorial treatment structure was to evaluate the effect of 2 light intensities, 7 fasting and 5 storage times on total protein (TP), uric acid (UA), and creatinine (CRE) concentrations in 2 blood fractions (serum vs. plasma) of broilers. Male Cobb 500 ($n = 140$) were grown to 42 d-of-age ($BW = 3.1 \pm 0.7$ kg), then, birds were redistributed into 7 replicate pens (10 birds per pen) per light intensity (5 and 20 lux) and reared to 45-d-of-age. Broilers were fasted for 1 h and then allowed to consume feed for 30 min prior to the initial blood collection (0 h). Blood was then collected from 1 of the 7 replicate pens from each environment (5 and 20 lux) via ulnar venipuncture using vacuum tubes with and without anticoagulant (5 mg NaF + 4 mg EDTA-K₃) at 2-h-intervals during the 12 h fasting period (0, 2, 4, 6, 8, 10 and 12 h). Serum and plasma fractions were collected following centrifugation and analyzed after 0, 15, 30, 60, and 120 d of storage in microtubes at -20 °C. Analysis was done using a spectrophotometry biochemical autoanalyzer (Flexor EL 200) with specific kits (Elitech) calibrated with ELICAL standards. Data were analyzed as a 1-way ANOVA by main effect with SAS PROC GLM with mean separation at $P < 0.05$ and polynomial regression was performed for fasting and storage time. Birds reared with 5 lux of light had greater concentrations of TP in both fractions as well as serum UA and plasma CRE compared with those at 20 lux ($P \leq 0.0295$). Fasting time did not alter TP in either fraction ($P \geq 0.2947$), but a cubic effect was observed on UA and CRE ($P < 0.0001$). Total proteins and UA increased linearly as storage time increased ($P \leq 0.0006$), while CRE decreased after 42 d of storage ($P < 0.0001$). Based on the regression analysis, fasting birds for no more than 5.3 h results in the fewest changes in these blood chemistry parameters. For analysis of TP, plasma can be stored for up to 120 d, while for serum, storage for less than 60 d is recommended. For UA and CRE, both fractions can be used, but immediate analysis is recommended after sample collection. Overall, these data shed light on the importance of sample collection

and storage strategies for biochemical analyses of blood metabolites in broiler chickens.

Key Words: broiler chickens; fasting; storage; light intensity; blood analysis

58 Impact of initial cell plating density of broiler chicken Pectoralis major muscle satellite cells on viability, proliferation, and heterogeneity. Caroline Gregg^{1*}, Brittany Wall¹, Joshua Flees¹, Charles Starkey¹, Jessica Starkey¹, ¹Auburn University, Auburn, Alabama, United States.

Muscle stem cells- also called satellite cells (SC)- signal in a contact-dependent manner making initial density of SC important for proper in vitro evaluation. Culture conditions for broiler chicken SC are not standardized in current literature. The objective was to evaluate increasing initial cell plating densities on proliferation, viability, and myogenic regulatory factor (MRF) heterogeneity of SC isolated from broiler chicken pectoralis major muscles (PM). Primary SC cultures were isolated from the PM of 9, 15, and 17-d-old male Yield Plus x Ross 708 broilers. SC were plated on triplicate, gelatin-coated, 1.8 cm²-wells at 4 initial densities (10,000, 20,000, 30,000, or 40,000 cells per well). SC were cultured in low glucose Dulbecco's Modified Eagle's Medium with 10% chicken serum, 5% horse serum, 1% antibiotic/antimycotic, and 0.1% gentamicin in a tri-gas incubator at 40 °C. At 48 and 72 h post-plating, adherent cells in parallel plates were detached with 0.25% trypsin. A Countess™ 3 Automated Cell Counter was used with trypan blue staining to assess viability and calculate doubling time (DT). At 96 h, 1 plate was stained using indirect immunofluorescence with a nuclear counterstain (DAPI) to detect SC expressing MRF paired box 7 (Pax7) and myogenic differentiation factor 1 (MyoD). Stained wells ($n = 12$ per replicate) were imaged using a Nikon Eclipse Ti2® inverted fluorescence microscopy system with Nikon Elements software. Images of 5 random fields were captured per well, and populations of Pax7+, MyoD+, and Pax7+:MyoD+ cells were enumerated and expressed on a mm² basis. Total Pax7+, MyoD+, and Pax7+:MyoD+ as well as MRF- (Pax7-:MyoD-) and MRF+ (Pax7+, MyoD+, Pax7+:MyoD+) were calculated and expressed as a proportion of total DAPI+ cells. The experiment was replicated with primary cultures from 3 independent groups of birds. Data were analyzed as a 1-way ANOVA using PROC GLIMMIX of SAS 9.4. Means were separated at $P \leq 0.05$ and tendencies were declared when $0.0501 \leq P \leq 0.10$. Cell viability was diminished 15.5% in cultures plated with only 10,000 cells per well ($P < 0.0001$). DT and proportions of MyoD+ and Pax7+:MyoD+ cells were not impacted by plating density ($P = 0.2360$). Yet, the pairwise mean comparisons revealed that plating density of at least 30,000 cells per 1.8 cm²-well results in increased proportions of Pax7+ compared with

10,000 and 20,000 cells per 1.8 cm²-well ($P \leq 0.0693$). In conclusion, plating primary broiler SC at a density of at least 30,000 cells per 1.8 cm²-well appears to best support both proliferation and progression toward differentiation in culture.

Key Words: broiler chicken; myogenic regulatory factor; proliferation; skeletal muscle satellite cell; cell density

59 In ovo feeding of probiotics changes the timing of gene expression in the small intestine during the first week post-hatch in broiler chicks. Sara Cloft^{1*}, Zehava Uni², Eric Wong¹, ¹*Virginia Tech, Blacksburg, Virginia, United States*, ²*The Hebrew University of Jerusalem, Rehovot, Israel*.

In ovo feeding into the amnion supplements the chick during the hatching process and fosters the transition to feed post-hatch. Previous research has established that nutrients provided in ovo hasten the maturation of the small intestine. Similarly, probiotics have been reported to promote immunocompetence in the first 7 days (d) post-hatch. This study aimed to clarify the effect probiotics have on the small intestinal maturation. On embryonic day (e) 17.5, commercial broiler eggs were punched with an egg piercer and then injected with 0.3ml of 10⁶ CFU of Primalac probiotic in 0.75% saline (Probiotic), 0.3 ml of 0.75% saline (Saline), or were not injected (Punch). All 3 segments of the small intestine were collected from chicks on e20, 1, 3, and 7 days post-hatch. Gene expression of the enterocyte marker Peptide transporter 1 (PepT1), and goblet cell marker Mucin-2 was examined by quantitative PCR. Log transformed data were analyzed by day using an ANOVA considering segment and treatment. On e20, the Probiotic group had increased expression of Mucin-2 and PepT1 mRNA in the ileum compared to the Punch group. Also, we observed that the Saline group at e20 had increased PepT1 mRNA in all intestinal segments compared to the Punch group though not as great as the Probiotic group ileal mRNA abundance. However, post-hatch there was no effect of in ovo injection on mRNA expression. Interestingly, the Punch group had Mucin-2 and PepT1 gene expression peaks in the ileum on 3 and 7d, respectively likely indicating the normal pattern of intestinal maturation. Previous research has shown that in ovo feeding stimulates the absorptive enterocyte to produce greater amounts of nutrient transporters such as PepT1. Additionally, it has been previously suggested that Mucin-2 may require antigenic stimulation within the intestine before it can be upregulated, which may explain why the Probiotic group was increased at e20. Therefore, these results present a case of altered timing of gene expression where in ovo feeding of probiotics causes earlier peaks of Mucin-2 and PepT1 than non-injected controls suggesting an enhanced intestinal maturation process and improved immunocompetence in agreement with previous studies.

Key Words: Probiotics; PepT1; qPCR; Muc2; In ovo Feeding

60 Chronic glucocorticoid administration induce sex differences in heterophil-to-lymphocyte ratio and increase cortisol levels in egg albumen in Pekin ducks. Victoria Tetel^{1*}, Sara Tonissen², Esther Oluwabenga¹, Gregory Fraley¹, ¹*Purdue University, West Lafayette, Indiana, United States*, ²*Purdue University, Olmsted Falls, Ohio, United States*.

Recent studies have indicated that cortisol may have physiological roles and that there are sex differences in the actions of both glucocorticoids (GC). During chronic stress, there is an initial increase in GC levels, but they then return to low, albeit not baseline, levels. The purpose of our study was to test the hypothesis that chronic treatment with low levels of either corticosterone or cortisol would alter HLR and organ morphometrics. Further, we wanted to determine if chronic treatment with either GC would elicit an increase in cortisol levels in egg albumen due to the recent findings that cortisol, not corticosterone, was localized to the albumen. To test our hypotheses, we implanted silastic capsules subcutaneously under propofol anesthesia. Capsules contained corticosterone, cortisol, or an empty capsule as controls and implanted on the back of the neck of adult ducks (N=5/sex/dose). On days 0, 7, and 14, blood serum, blood smears, body weights, were collected. Eggs were collected daily for egg quality assessments. After 2 weeks, ducks were euthanized and body, liver, and spleen weights, and the number of active follicles were recorded. Albumen glucocorticoid levels were assessed using mass spectrometry. Data were measured using a 2- or 3-way ANOVA as appropriate and *post hoc* with Fishers PLSD. No treatment elicited differences in egg quality measures or body weight compared to controls. After two weeks, corticosterone, but not cortisol, treatment elicited a significant increase in serum corticosterone (1.6 +/- 0.12 ng/ml) compared to controls (0.7 +/- 0.09 ng/ml) in both sexes. Cortisol treatment elicited a significant ($p < 0.05$) increase circulating cortisol (121.6 +/- 5.17 pg/ml) as did treatment with corticosterone (47.7 +/- 6.34 pg/ml) compared to control levels of cortisol (19.3 +/- 4.25) in both sexes. Relative spleen weights were significantly ($p < 0.05$) reduced in hens following corticosterone but not cortisol treatment. No other organs showed any significant differences among the treatment groups. Both GCs elicited a significant ($p < 0.001$) increase in HLR in hens, but not drakes, at all time points over the 2 week treatment period compared to controls. Chronic treatment with cortisol, but not corticosterone, elicited a significant ($p < 0.01$) increase in egg albumen cortisol levels compared to other groups. Corticosterone was not detected in any albumen samples. Our results suggest that glucocorticoids elicit differential effects and although corticosterone has been stated to be the predominant GC in avian species, cortisol may provide critical information to further understand and improve welfare.

Key Words: Eggs; Corticosterone; Epigenetics; Cortisol; Breeder ducks

61 Chronic heat stress decreases egg quality, increases cortisol levels in egg albumen, and affects fertility in Pekin ducks. Esther Oluwagbenga^{1*}, Jenna Schober¹, Victoria Tetel¹, Sara Tonissen¹, Gregory Fraley¹, ¹Purdue University, West Lafayette, Indiana, United States.

Heat stress (HS) has detrimental effects on production, health, and welfare of poultry. The goal of our experiment was to test the hypothesis that HS would alter welfare, egg quality (EQ), and morphometrics of breeder ducks. Furthermore, we wanted to test if HS would increase cortisol levels in egg albumen due to recent exciting findings that cortisol, not corticosterone, is isolated in albumen. To test our hypothesis, adult Pekin ducks were randomly assigned to two different rooms at 85% lay with 80 hens and 20 drakes per room. Baseline data including body weight (BW), body condition scores (BCS; footpad quality, eyes, nostrils, feather cleanliness and feather quality), and egg production/quality were collected the week preceding heat treatment. Ducks were subjected to cyclic HS of 35°C for 9h/day and returned to 29.5°C for the remaining 15h/day for 3 weeks while the control room was modified to an industry-standard temperature of 22°C. Eggs were collected daily and body weights were taken on days 0 and 21 relative to the onset of heat treatment. BCS were collected weekly. Eggs for quality assessment were collected weekly and albumen glucocorticoid (GCs) levels were assessed using mass spectrometry. After 3 weeks, 10 hens and 5 drakes were euthanized per group using pentobarbital. BW, weights of the liver, spleen, and the number of maturing follicles were recorded. Data analyses were done by 2- or 3-way ANOVA as appropriate with a Tukey-Kramer *post hoc* test. BCS were analyzed using a chi-squared test. A $p \leq 0.05$ was considered significant. Feather quality ($p < 0.01$), feather cleanliness ($p < 0.001$) and footpad quality ($p < 0.05$) increased significantly in the HS group compared to controls. HS elicited a significant ($p < 0.05$) decrease in egg production and number of fertile eggs upon candling at 10 days of incubation. Hens in the HS group showed a significantly decreased BW ($p < 0.001$), relative spleen weights ($p \leq 0.05$), and number of ovarian follicles ($p < 0.05$) compared to controls. There is a time-dependent decrease in shell thickness at weeks 1 and 2 in the HS group but no difference at week 3 compared to the baseline ($p < 0.001$). Shell weight decreased significantly at week 1 only ($p < 0.05$) compared to controls. Yolk weight decreased significantly at week 3 ($p < 0.01$) compared to controls. HS elicited a significant increase in albumen cortisol levels at week 1 ($p < 0.05$) and week 3 ($p < 0.05$). HS elicited a selective deposition of cortisol, not corticosterone, in the egg albumen confirming previous results by others. Further, HS has a time-dependent effect on egg quality parameters. The impact of HS on egg quality and cortisol levels suggests further research is needed on the effects of HS on the F₁ generation.

Key Words: Pekin duck; Welfare ; Glucocorticoids; Heat stress; Egg quality

62 Impact of rearing environments on growth trajectories and sexual maturation in layer chickens. Mohammad Bahry^{1*}, Charlene Hanlon¹, Sierra Schaus¹, Clara Ziezold¹, Gregoy Bedecarrats¹, ¹University of Guelph, Guelph, Ontario, Canada.

Our recent studies showed metabolic triggers can independently initiate sexual maturation when a threshold is met. Moreover, previous research showed that growth and body composition of layer pullets are influenced by rearing environments. Thus, this study aimed to determine whether different housing systems (HS) affect the metabolic threshold required to activate the reproductive axis in layers. Day-old Lohman LSL lite (W, n=224) and Lohman brown (Br, n=224) chicks were randomly assigned to either Conventional cages (CC, n=18), Aviary 1 (AV1, n=52, confined for the first 6 weeks), Aviary 2 (AV2, n=64, access to vertical space for the first 6 weeks) or Aviary 3 (AV3, n=90, open concept for the first 6 weeks) on a 2 x 4 factorial arrangement of treatments. At 6 weeks of age (woa) all systems (except CC) were opened to provide access to a litter area. All hens received *ad-lib* feed and lighting was scheduled as per the breeder's recommendation. Body weight (BW) and blood samples (n=10 per group) were collected throughout the study and estradiol (E2) profiles were determined. At 16 and 23woa a subset of birds were euthanized (n=6) and carcass composition was determined by Dual-energy X-ray absorptiometry (DEXA). Statistical analyses were performed using the MIXED procedure in SAS. Results show that Br pullets were heavier ($P < 0.001$) than W and pullets in CC had higher BW compared to AV systems for both strains. The impact of HS was more pronounced for Br than W with pullets in AV1 and AV3 displaying the lowest BW ($P < 0.001$). The HS impacted sexual maturation in a strain-dependent manner with the E2 peak delayed by 2 weeks for W in AV3 and 3 weeks for Br in AV1 ($P < 0.001$). Egg-laying was also delayed for W in AV1 and AV3 ($P < 0.001$). Regardless of HS, Br started laying 1 week before W. Results of DEXA show that HS had a significant impact on carcass fat ($P < 0.001$) with pullets in CC reaching 20% fat ratio at 16woa for both strains while all 3 AV systems resulted in levels below 16%. At 23woa, the fat ratio in CC remained unchanged while levels in AV systems caught up, except for W in AV1. Bone mineral density was significantly reduced ($P < 0.001$) in CC pullets at 16woa, however, at 23woa, no difference was observed between treatments. At 16woa, W pullets displayed lower ($P < 0.001$) bone mineral density than Br. In summary, the results show that rearing pullets in CC results in increased body weight and carcass fat percentage while lowering bone mineral density at 16woa. The type of aviary system also had an impact on body weight and body composition, however, a significant concomitant impact on egg production was only observed in W birds.

Key Words: Layers; Egg production; Housing system; Sexual maturation; Growth trajectory

63 *In vivo* tibiotarsal axial stiffness is unaffected by genetic strain and rearing housing. Isabela Vitienes^{1*2}, Seyedmahdi Hosseinitabatabaei^{1,2}, Alice Bouchard^{1,2}, Catherine Julien^{1,2}, Ana Rentsch³, Tina Widowski³, Russell Main⁴, Bettina Willie^{1,2}, ¹McGill University, Montreal, Quebec, Canada, ²Shriners Hospitals for Children Canada, Montreal, Quebec, Canada, ³University of Guelph, Guelph, Ontario, Canada, ⁴Purdue University, West Lafayette, Indiana, United States.

Bones adapt to maintain structural integrity by sensing mechanical strain (deformation) occurring during daily activity and responding by forming or resorbing bone. Bone stiffness therefore affects how bones sense and adapt to load-bearing activity. We aimed to determine whether a pullets' genetic strain and physical activity history, determined by rearing housing complexity, affects *in vivo* axial stiffness of the tibiotarsal midshaft. Across three consecutive flocks, a total of 7,128 Lohmann Brown-Lite (B) and LSL-Lite (W) pullets were reared in either conventional cages (CC) or two aviary rearing systems with varying levels of complexity (Low, L; High, H) until 16 woa. We sampled 42 birds (n=7/strain/housing) and surgically instrumented their right tibiotarsi with strain gauges at the anterior, medial, and posterior midshaft surfaces. Axial compressive loads were applied to the right hindlimb at a range of peak load levels (-30 to 220N) using a controlled loading device while the engendered strains were measured, and the mean *in vivo* axial stiffness at each bone surface was derived for each chicken. Data were analyzed by ANOVA and Tukey post-hoc testing, with significance at $p \leq 0.05$. There was no effect of genetic strain, rearing housing, or their interaction on axial stiffness at any of the surfaces measured. Axial stiffness averaged $-0.63\text{N}/\mu\epsilon$, $0.06\text{N}/\mu\epsilon$, and $0.11\text{N}/\mu\epsilon$ at the anterior, medial, and posterior surfaces respectively. Axial stiffness magnitude (irrespective of strain direction) differed significantly across surfaces measured, with the anterior surface being stiffer than medial and posterior surfaces, whereas medial and posterior axial stiffness magnitude did not differ. These data elucidate the relationship between applied axial load and the strain this load produces at the tibiotarsal midshaft. This allows us to identify -170N as a target load for *in vivo* controlled loading experiments, capable of engendering anabolic medial and posterior strains up to 15 times those occurring during ground locomotion, which we report to be $-187\mu\epsilon$ and $-293\mu\epsilon$ respectively¹. In addition, we recently reported that aviary rearing enhanced bone mass and microstructure in B and W pullets respectively², adaptations known to increase bone stiffness. The observed comparable stiffness in aviary and CC groups suggests that changes in bone morphology or tissue material properties may be counteracting the effects of bone mass and microstructure to maintain *in vivo* tibial stiffness across rearing housing groups. Further work is

ongoing to characterize bone morphology, material properties, and the mechanoresponse in these groups.

1) Vitienes et al. PSA 2022 under review.

2) Vitienes et al. PSA 2021

Key Words: Genetic strain; Skeletal mechanobiology; Bone adaptation; Mechanical strain; Rearing housing

64 Changes in acid-base status and circulating ionized calcium and phosphorus levels in laying hens during early and peak production. Micaela Sinclair-Black^{1*}, Alejandra Garcia Mejia¹, Rosalina Angel², Bibiana Jaramillo⁴, Xabier Arbe³, David Caverio³, Laura Ellestad¹, ¹University of Georgia, Athens, Georgia, United States, ²University Of Maryland, College Park, Maryland, United States, ³H&N International, Bangkok, Thailand, ⁴Iluma Alliance, Durham, North Carolina, United States.

In laying hens, homeostatic regulation of blood ionized calcium (iCa) and phosphorus (P) is crucial for maintaining skeletal health and eggshell quality. During egg formation, calcium from feed and iCa mobilized from bone are used for shell calcification; bone mobilization also releases P into the blood. Acid-base status can affect levels of iCa, with lower pH increasing iCa due to its reduced interaction with binding proteins. Therefore, changes in acid-base status may have consequences for skeletal health and eggshell integrity, factors that become increasingly important as hens age. This research evaluated fluctuations in laying hen blood chemistry at 25 and 43 weeks of age. Blood was collected from the brachial wing vein of 96 Nick Chick hens (H & N North America) at four time points during the 24-hour egg formation cycle [1.5, 6, 15 and 21 hours post oviposition (HPOP); n=12 hens/time point]. Aspects of blood chemistry including total P (tP), iCa, pH, and bicarbonate (HCO_3^-) were measured immediately using VETSCAN VS2 Avian/Reptilian and iSTAT CG8+ cartridges (Zoetis). Data were analyzed by a two-way ANOVA, and means were compared using Fisher's LSD test when the ANOVA indicated significance ($p \leq 0.05$). While no interactive effects were noted between hen age and HPOP ($p > 0.5$), main effects of age and HPOP were observed. As hens aged from 25 to 43 weeks, iCa decreased ($p \leq 0.05$). At both ages, iCa decreased between 1.5 and 6 HPOP, was further reduced at 15 HPOP, and remained low through 21 HPOP ($P \leq 0.05$), likely reflecting periods of bone remineralization and eggshell calcification, respectively. Blood tP levels were unaffected by age ($p > 0.05$); however, they were influenced by the stage of egg formation. At 1.5 HPOP, tP levels were low and, following this, increased steadily through 15 HPOP after which they plateaued ($P \leq 0.05$). These changes could be indicative of excess P remaining from bone breakdown as iCa was utilized for eggshell formation between 6 and 21 HPOP. Blood pH was higher in 25-week-old hens ($p \leq 0.05$) and, at both ages, pH increased between 1.5 and 15 HPOP ($p \leq 0.05$) and remained elevated through 21 HPOP. Levels of HCO_3^- were higher at 43 weeks and increased linearly from

1.5 to 15 HPOP at both ages ($p \leq 0.05$); however, HCO_3^- then decreased from 15 to 21 HPOP ($P \leq 0.05$). Taken together, these results suggest that as hens progress through the peak production period, metabolic shifts might occur that influence iCa availability for shell formation and bone mineralization. Strategies aimed at managing these shifts to optimize iCa availability and utilization could positively impact skeletal integrity and egg quality, particularly during an extended production cycle lasting through 90 weeks of age.

Key Words: calcium; phosphorus; layers; blood; acid-base status

65 Changes in gene expression in shell gland and kidney related to the onset of egg production in commercial laying hens. Rahab Garcia^{1*}, Micaela Sinclair-Black¹, Lyssa Blair¹, Rosalina Angel², Bibiana Jaramillo³, Xabier Arbe⁴, David Caverio⁴, Laura Ellestad¹, ¹University of Georgia, Athens, Georgia, United States, ²University Of Maryland, College Park, Maryland, United States, ³Iluma Alliance, Durham, North Carolina, United States, ⁴H&N International, Bangkok, Thailand.

At the onset of egg production in laying hens, changes in pathways regulating calcium (Ca) and phosphorus (P) uptake and utilization for shell mineralization must occur, and many of these changes are regulated by vitamin D₃. The objectives of this study were to 1) determine changes in gene expression associated with vitamin D₃ action and Ca uptake in kidney and shell gland prior to and after onset of lay and 2) evaluate effects of dietary supplementation with 1 α -hydroxycholecalciferol [1 α (OH)D₃] on these changes. Kidney and shell gland were collected from Nick Chick hens (H & N North America) fed developer diet prior to the onset of lay (18 wks; n=8) and at early peak production (31 wks) from hens fed either a control or 1 α (OH)D₃-supplemented onset diet for 14 wks prior to sampling (n=8/diet). Levels of mRNA were measured by RT-qPCR, and target genes were normalized to glyceraldehyde 3-phosphate dehydrogenase in kidney and 18S ribosomal RNA in shell gland. Data were analyzed by one-way ANOVA followed by the test of least significant difference when ANOVA indicated significance ($P \leq 0.05$). Changes in mRNA expression were not influenced by diet, however several age effects were found. In kidney, mRNA expression of vitamin D receptor (*VDR*) did not differ between 18 and 31 wks; however, the *VDR* heterodimeric partners retinoid-X-receptor alpha (*RXR α*) and gamma (*RXR γ*) were higher at 31 wks ($P \leq 0.05$). Two transporters required for Ca uptake, plasma membrane Ca ATPase and transient receptor potential vanilloid 6, and the cytoplasmic Ca chaperone calbindin (*CALBI*) all increased at 31 wks in kidney ($P \leq 0.05$). Expression of three P transporters, sodium/phosphate cotransporter 1 and inorganic phosphate transporters 1 (*P₁T-1*) and 2 (*P₁T-2*) also increased at 31 wks ($P \leq 0.05$), with *P₁T-1* having a dramatic 10-fold increase in expression. In shell gland, expression of *VDR* increased at

31 wks ($P \leq 0.05$), but *RXR α* and *RXR γ* did not differ between ages. Carbonic anhydrase, required for formation of bicarbonate that can be used for calcium carbonate deposition in eggshell, increased at 31 wks in shell gland ($P \leq 0.05$), and *CALBI* showed a remarkable 35-fold increase at 31 wks in this tissue ($P \leq 0.05$). Like kidney, expression of *P₁T-1* and *P₁T-2* was greater at 31 wks in shell gland ($P \leq 0.05$), with *P₁T-1* exhibiting a 9-fold increase. Although unaffected by dietary 1 α (OH)D₃, changes in gene expression related to the physiological regulation of Ca and P metabolism necessary for eggshell formation at the onset of lay were demonstrated. These include an increased capacity for mineral reabsorption or excretion in the kidney and Ca utilization by the shell gland. In both tissues, *P₁T-1* appears to be the primary transporter mediating P flux.

Key Words: kidney; vitamin D₃; shell gland; calcium transport; phosphorus transport

66 Utilizing the 3D environment to facilitate learning of complex neural pathways in the bird brain. Parker Straight^{1*}, Paul Gignac², Wayne Kuenzel¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²University of Arizona, Tucson, Arizona, United States.

Neuroanatomical pathways are challenging to study often due to the limit of techniques used to visualize their anatomical and physiologic characteristics. In many studies, a neural pathway is presented using 2D representations for structural connectivity. A problem is deciding which of three planes: coronal, sagittal, or horizontal is best for visualizing the pathway's components clearly and spatially precise for those wanting to learn and utilize that information. A 3D environment would be imperative to solving this issue. We therefore attempted to develop a means of accurately presenting detailed anatomical structures within the 3D regions they occurred, including dendrites, cell bodies, axons and synapses. It is our hope that accurate, spatial representations of neural pathways will result in learning specific structures, their subdivisions, and their spatial organizations. Recent technological advancements, such as diffusible iodine-based contrast-enhanced computed tomography (diceCT), has allowed for nondestructive visualization of an appropriately fixed brain. In other words, it allows one to immunostain the entire brain, and visualize any of the three planes without damaging the specimen. We have chosen the visual tectofugal pathway in the brain as it is one of the most well studied systems that seems to have much disparity in the anatomical organization and connectivity. This pathway begins in the eyeball with retinal ganglion cells projecting to the optic tectum which in turn sends projections to a thalamic nucleus. This thalamic nucleus then projects to a region of the forebrain, completing the ascending pathway. For our investigation we employed two techniques: stacked series of histologic sections of chicks, and diceCT scanning of an entire chick brain. For histological sections, we used 3 birds to produce series of coronal, sagittal, and horizontal

sections stained with Nissl (cell bodies revealed) and Giemsa (fiber tracts revealed). Sections were imported into Brainmaker (Microbrightfield Biosciences), a software that stacks image sequences and produces a 3D model. For our diceCT investigation, we rendered the eyeball and brain within the skull of the bird. Post model processing was necessary to incorporate detailed 2D images in the appropriate plane of the 3D environment. Using the diceCT scanned brain, histological image stacks, and 3D editing

software, we created an interactive 3D model of the avian visual tectofugal pathway. The combination of histochemical sections with diceCT 3D modeling is necessary when detailed anatomical and spatial organization of complex pathways such as the tectofugal visual system are desired.

Key Words: 3D modeling; vision; diceCT; avian neural pathway; image processing

Student Competition: Extension and Instruction

67 Poultry industry and welfare-focused survey responses over four semesters of undergraduate students in an introductory poultry science course.

Meaghan Meyer^{1*}, Elizabeth Bobeck¹, ¹*Iowa State University, Ames, Iowa, United States.*

Understanding how science-based instruction may alter views of the poultry industry is an important part of improving university instruction as well as engaging with consumers. The research objectives were to quantify the effects of student background, career interests, and a semester of science-based education on opinions of current issues faced by the poultry industry. Undergraduate students enrolled in a Poultry Science course at Iowa State University between 2018-2021 were surveyed at the start and end of the semester as part of a four-year study. Surveys were collected anonymously on paper with self-assigned code names used only to identify and compare responses before and after taking the course. Students who opted to take the survey were first asked to answer three demographic questions regarding 1) their level of farm-animal experience, 2) sex, and 3) current career goals (poultry industry, other livestock industry, or non-animal industry). The remainder of the survey consisted of 16 “poultry issue statements” where students were directed to mark a vertical line on a 130mm horizontal line indicating their level of agreement/disagreement with each statement. Post-survey collection, the 130mm line was separated into 5 sections for categorization and analysis: responses within 0- 20% indicated strongly disagree, 21-40% indicated disagree, 41-60% indicated neutral, 61-80% indicated agree, and responses within 81-100% indicated strongly agree. The number of students surveyed varied slightly by year based on enrollment and number of students present in class for both the pre- and post-course survey. Numbers from 2018-2021 were as follows: 29, 27, 26, and 26 students. Responses were analyzed using Proc Mixed in SAS Version 9.4 (Cary, N.C., U.S.) with a Tukey-Kramer adjustment for all pairwise comparisons using main effects obtained from demographic questions (livestock experience, sex, and career industry goals) as well as the main effects of education (pre- or post-instruction) and year. Responses to various issue statements were affected by students’ livestock experience ($P<0.05$; 6 out of 16 statements affected), sex ($P<0.05$; 5 out of 16 statements), and ultimate career goals ($P<0.05$; 3 out of 16 statements). Further, in 3 out of 16 poultry issue statements, the year of instruction affected student response ($P<0.05$). Pre- versus post-education responses differed significantly in 6 out of 16 statements ($P<0.05$). These data indicate that student background, sex, and career interests impact opinions of relevant topics in the broiler and layer industries. Knowledge provided through education as well as the year the course was taken over consecutive semesters altered student opinions.

Key Words: animal welfare; education; poultry science ; poultry industry; undergraduate teaching

68 Development and publication of interactive online poultry embryology education modules. Ashley Bigge^{1*}, Josephine Foley¹, Sheila Purdum¹, ¹*University of Nebraska-Lincoln, Lincoln, Nebraska, United States.*

Online education for K-12 classroom learning has been growing in recent years, especially in the wake of the COVID-19 pandemic. The Hippology Academy series from eXtension Horses, Inc. has found great success in teaching through the use of online education materials, but such a program did not previously exist for poultry. Our primary objective was to create accessible and affordable materials appropriate for a variety of age groups, from 4-H youth to adults. Priority was given to developing an online embryology module. Embryology yields a high degree of interest in K-12 science classes and Nebraska 4-H poultry project enrollment. The modules were designed to also incorporate information relevant to backyard flock owners, and increase familiarity with commercial practices and standards. The embryology course design model included one main lesson module accompanied by two supplemental topics. We determined the following development cycle to be effective. Modules were developed using Articulate 360 software, particularly Storyline 360. These drafts were reviewed and edited until a final draft was ready for external review by county extension educators prior to publication. The ideal platform for publication needed to be as accessible as possible, support the files we would need hosted, and have the ability to collect analytics. We chose the online eXtension Foundation Campus site which uses a Moodle server as its learning management system. Several means of assessment were incorporated within each course including a pre-module quiz and embedded questions within each lesson. The modules are self-paced, so the learner can freely retake assessments until they attain a grade they are satisfied with. The results of all assessment attempts are reported on eXtension, along with other data such as course and activity views, interactions, and completion. The first embryology module series was released early March 2022, coinciding with the spring arrival of fertile eggs and chicks. Social media, email, and extension newsletters were among the methods of promotion used. As of April 15, 2022 the embryology course summary has been viewed 87 times, has 2 currently enrolled students, and 1 course completion. We expect learner engagement to increase over the coming months, especially as we continue to add to our content library. Future module developments will cover other 4-H and FFA projects, such as poultry judging.

Key Words: education; online; learning management system; course design; embryology

69 Validation of iQ® sensing rodent traps for efficient and effective rodent management. Torey Fischer^{1*}, Jason Fields¹, Steve Von Haden², Seth Keller², Darrin Karcher¹, ¹*Purdue University, Lafayette, Indiana, United States*, ²*Motomco, Windsor, Wisconsin, United States*.

Rodents can cause a multitude of problems in poultry houses including loss of feed, building damage and decrease in other production values. Rodents are also known to carry certain bacterial diseases that impact poultry, such as salmonellosis and mycoplasmosis. Technologies involving Bluetooth® and automated data uploads have evolved into a new approach to rodent management. These traps come with a battery, sensor and antenna, which are all water and weather-proof. The sensors collect timestamps of rodent activity and communicate via Bluetooth iQ® Bell Sensing Technologies® (BST) App. The objectives of this trial were to validate the use of iQ rodent monitoring products, powered by BST, for effectiveness compared to normal traps, changes on time and labor, and effectiveness to reduce rodent populations in a poultry facility. A variety of traps were placed in three different buildings at the Purdue Poultry Unit. Comparisons of iQ to traditional products allowed us to look at service time differences in the first two buildings. Building 1 had 12 Tomcat® Live Catch iQ rodent traps, 24 Tomcat® Bullet® iQ rodent bait stations, 12 traditional live catch, and 24 traditional rodent bait stations placed throughout the barn. The live catch traps were serviced 9 times over a 3-month period and the bait stations

were serviced 11 times over a 4-month period. Building 2 had 26 Bullet iQ Rodent Bait Stations and 26 traditional bait stations (non-iQ) placed and serviced 11 times over a 4-month period. Building 3 had 18 Bullet iQ Rodent Bait Stations placed and serviced 11 times over a 4-month period. The data collected were analyzed using a paired t-test in R. The trial results reported a 49%-time savings ($p=0.003$) when using iQ live trap products compared to manually checking live-catch traps in Building 1, in addition to a reduction in rodent activity in all poultry buildings. The average reduction in rodent activity across the buildings was about 98.6% ($p=0.003$). The results found higher catch rates using iQ products in live catch devices over a 3-month period ($p=0.0006$). These iQ traps and bait stations were able to provide data about rodent movement in the buildings. Data collected by the iQ products is automated and can be easily accessed and sent to any Bluetooth enabled smart phone or tablet, which is often more accurate than manual pen and paper recording with traditional traps. New technologies like these could be useful for laying hen facilities to meet FDA compliance. These results demonstrate that a new approach for rodent management could be valuable to poultry producers and other livestock species in more efficiently and effectively managing rodents.

Key Words: poultry; technology; rodent; live traps; bait stations

Student Competition: Management and Production

70 Effect of body weight trajectories on broiler breeder efficiency, fat deposition, and uniformity during rearing. Thiago Noetzold^{1*}, Martin Zuidhof¹, ¹University of Alberta, Edmonton, Alberta, Canada.

A study was designed to analyze the effect of broiler breeder pullet BW on efficiency, fat deposition, and uniformity. We hypothesized that early BW growth would increase BW, abdominal fat pad, and FCR; and that BW uniformity would be higher in a precision feeding (PF) system compared to a conventional (CON) system. Two concurrent PF studies plus a standard CON system were implemented to 21 wk of age. A total of 678 day-old Ross 308 broiler breeder females were randomly assigned to various BW trajectories. In both PF studies the BW trajectories were designed using a 3-phase Gompertz model ([endif]-->, where g_i was the amount of gain occurring in phase i ; b_i was the rate of maturing of phase i ; I was the inflection point of phase i ; and t was age (wk). Both PF experiments had a 2 x 6 factorial treatment arrangement. Experiment 1 (Exp1) trajectories had 2 levels of g_1 (prepubertal growth): standard, where g_1 was equivalent to the phase 1 growth estimated from the breeder-recommended (BR) target, or a 20% shift from g_2 (pubertal growth) to g_1 ; and six I_2 ranging from 15 to 23 wk. Experiment 2 (Exp2) trajectories had two rates b_2 : standard or 50% greater; and six shifts of growth from g_2 to g_1 , ranging from -10 to 40% of BR g_2 . CON birds were grown to the BR target BW. Body weight and feed intake (FI) of CON birds were recorded once per week. Feed intake and BW were recorded daily, and abdominal fat pad weight was recorded at 21 wk of age in the PF system. FCR and coefficient of variation (CV) for BW were calculated weekly. PF experiments were submitted to 3-way ANOVA, with g_1 and I_2 (Exp1) or b_2 and g_2 to g_1 shift (Exp2), and age as sources of variation. ANCOVA was used to analyze abdominal fat pad data. Comparisons among CON and PF systems were made using a 2-way ANOVA with feeding system and age as sources of variation. BW differed according to the respective BW trajectories in both PF system experiments ($P < 0.05$). In Exp1, I_2 did not affect FCR, but standard g_1 pullets had a lower FCR (3.77) compared to the 20% shift from g_2 to g_1 (3.85; $P < 0.05$). In Exp2, b_2 did not affect FCR, but FCR increased as gain was shifted from the pubertal to prepubertal phase ($P < 0.05$). At 21 wk of age, fat pad increased 0.22% per wk that I_2 was advanced in Exp1 ($P < 0.05$), but fat pad did not differ in Exp2. BW CV was 0.9% in the PF system compared to 18.4% in the CON system ($P < 0.05$). In summary, early BW gain increased FCR, and the PF system yielded substantial uniformity improvement.

Key Words: broiler breeders; multiphasic growth; pubertal; prepubertal; precision livestock feeding

71 Bedding Management for Suppressing Particulate Matter in Cage-free Layer Houses. Ramesh

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Cage-free (CF) hen houses tend to have high levels of particulate matter (PM) due to presence of bedding/litter floor and birds' activities of perching, dustbathing, and foraging on it. Optimizing bedding management has potential to suppress PM levels. The objective of this study was to test the effect of top application of different new bedding materials on PM levels. This study was conducted in four identical CF layer rooms/facilities. Each CF aviary room consisted of four sections with 576 hens in total (144 hens per section; usable floor space of 1,132 cm²/hen. Fine shavings (FS), large flake shaving (LFS), and aspen wood chips (AWC) were top spread on the surface of original litter evenly in the treatment rooms at 20% volume of original litter floor (initial litter depth was 4.8±0.8, 4.9±1.0, and 4.7±0.9 cm in FS, LFS, and AWC room), respectively. The fourth room (litter depth was 4.6±0.6 cm) was used as the control without new bedding materials. Prior to the test of new bedding materials, the moisture content of bedding materials was measured as 17.2±1.9%, 18.5±0.8%, 17.1±2.3%, and 17.8±1.3% in control, FS, LFS, and AWC room, respectively. The PM concentrations were measured with TSI DustTrak-8533 sensors in the center of each section at 0.35 m height above the litter floor manually twice a week (9-10 am in the morning) over six weeks. Meanwhile, air temperature (T) and relative humidity (RH) were monitored continuously with HOBO sensors. The PM levels in the treatment and control rooms were analyzed with two-way ANOVA in JMP Pro-16. The difference was considered significant at $p < 0.05$. Results show that the top application of new bedding materials had significant effect on PM reduction ($p < 0.01$). The PM concentrations in the AWC room (i.e., 0.69 mg m⁻³-PM_{2.5}, 1.67 mg m⁻³-PM₁₀, and 2.67 mg m⁻³-TSP-total suspended particles) were lower than the control room (i.e., 0.95 m⁻³-PM_{2.5}, 2.32 m⁻³-PM₁₀, and 3.74 mg m⁻³-TSP) by 27.3, 28.3, and 28.7%, respectively. Reductions on PM_{2.5}, PM₁₀, and TSP levels in SF and LFS were similar (13.3-15.8%). For litter quality post-treatment, the moisture content was 18±2.5, 20±2.7, 20.6±2.4, 19.7±3.7% in the control, FS, LFS, and AWC room, respectively. The control room had drier litter than three treatment rooms ($p < 0.05$). The four rooms had similar air T/RH during the test (i.e., 23.1°C/65%, 22.8°C/67%, 23°C/65%, 23°C/66% in control, FS, LFS, and AWC room, respectively). Findings of this study show that topping new bedding on old litter is a potential strategy for suppressing dust in CF houses. Further studies such as the effect of different ratios of new bedding on PM control, the cost

analysis, and the verification tests in commercial CF houses are warranted.

Key Words: animal welfare; Egg production; litter quality; bedding materials

72 The effects of preening cups on production and body condition in grow-out Pekin ducks. Jenna Schober^{1*}, Mallory Swanson¹, Victoria Tetel¹, Esther Oluwagbenga¹, Drew Frey², Heidi Parnin², Marisa Erasmus¹, Gregory Fraley¹, ¹*Purdue University, Lafayette, Indiana, United States*, ²*Culver Duck Inc., Middlebury, Indiana, United States*.

Previous studies found that open water access increased litter moisture that leads to decreased production and increased mortality in commercial Pekin ducks. Other studies have found that open water access allows for natural preening behaviors and improved production. Preening cups have been suggested as an alternative to open water that would allow ducks to express preening behaviors. We set out to test the hypothesis that preening cups at a much higher density than utilized in industry would not have detrimental effects on the ducks or their environment. To test our hypothesis, 260 grow-out ducks were obtained from a commercial hatchery. Ducks were evenly and randomly placed into 2 pens in each of 2 rooms at Purdue's research farm. Ducks were housed to closely approximate industry standards for density. Preening cups were placed 1 per pen on Day 18 of life. The control pens only had nipple lines (8 ducks per nipple). The study was repeated with the preening cup and control pens swapped giving a final N = 4 pens per treatment. Ammonia levels were recorded in 3 places per pen weekly. Body weights of 30 ducks per pen were recorded weekly. Body condition scores were assessed on 50 birds per pen weekly. On Day 18 and Day 40, 5 ducks per pen were euthanized using FatalPlus (pentobarbital 396 mg/ml/kg) and their spleens, bursa, liver, and uropygial glands were weighed. Body condition scores were analyzed using Wilcoxon test and all other data were analyzed using a Student's t-test. The pen was the statistical unit for all data. No significant differences were observed in body weight at any age, nor in growth rates between groups. Organ weights were analyzed relative to body weights, however no significant differences were observed. No significant differences were observed between the two groups for any body condition score; however, feather scores were higher (thus worse, $p = 0.06$) in ducks with preening cups compared to controls. No significant differences were observed in any production, environmental, or necropsy data ($p > 0.1$). Previous studies have suggested that ducks raised without open water sources are not able to preen and thus show greatly reduced uropygial gland size; however, with the preening cups used in this study, no differences were found, suggesting that open water is not necessary to maintain feather condition and uropygial gland size. Our study shows that even at a very high density of ducks per

preening cup, their presence does not adversely affect the ducks or their environment.

Key Words: open water; preening

73 The Effect of Stocking Density on Commercial White Egg Layer Physical Egg Quality. Benjamin Alig^{1*}, Kenneth Anderson², Ramon Malheiros², ¹*North Carolina State University, Wake Forest, North Carolina, United States*, ²*North Carolina State University, Raleigh, North Carolina, United States*.

Stocking density remains a major welfare concern for commercial egg producers, due to the perception by consumers about excess stress on hens. Furthermore, many consumers also claim that eggs from these high stress environments are lower in quality. Therefore, it is important to validate these claims and determine if stocking density can affect physical egg quality. We hypothesized that higher densities would have a negative effect on egg quality. A total of 720 hens in five different density treatments consisting of 6 birds (208 in²/bird), 9 birds (139 in²/bird), 12 birds (104 in²/bird), 15 birds (83 in²/bird) and 18 birds (69 in²/bird) were examined in colony cage environments. Shaver whites were used for this study. There were six replicates represented by 2 cages of each treatment in colony cages measuring 48 in by 26 in. The laying cycle began at 17 weeks of age with a base period of 4 weeks for feed weigh backs and egg quality measurements and lasted for 12 periods. Physical egg quality parameters (shell color, yolk color, Haugh unit, albumen height, shell strength and vitelline membrane strength) were measured during periods 2, 4, 6, 8, 10 and 12. Each test utilized six eggs per replicate. Statistical analysis was performed utilizing JMP 15.2 and Tukeys HSD for multiple comparisons. The effects were density, period and the interaction and a p value < 0.05 was per considered significant. Overall, we found that stocking density did not have a statistically significant effect on the afore mentioned quality parameters measured. For example, shell strength measured 5.13kg/mm, 5.19kg/mm, 5.18kg/mm, 5.05kg/mm and 4.98kg/mm with a standard deviation of 0.062kg/mm for 6, 9, 12, 15 and 18 birds per cage, respectively. Haugh unit measured 89.2, 89.9, 90.3, 90.0 and 89.6 with a standard deviation of 0.40 for 6, 9, 12, 15 and 18 birds per cage respectively. Finally, vitelline membrane strength measured 2.15g/mm, 2.19g/mm, 2.09g/mm, 2.17g/mm and 2.76g/mm with a standard deviation of 0.26g/mm for 6, 9, 12, 15 and 18 birds per cage respectively. In conclusion, we must reject our hypothesis that higher densities yield poor quality eggs, and accept that stocking density does not affect physical egg quality of white egg laying hens.

Key Words: Stress; Egg Quality; Stocking Density; Egg Layers; Haugh Unit

74 Effectiveness of ventilation shutdown in an aviary housing system. Andrea Mendoza^{1*}, Shawna Weimer², Zachary Williams³, ¹*Michigan State University, Lansing,*

Michigan, United States, ²University of Arkansas, Fayetteville, Arkansas, United States, ³Michigan State University, Holt, Michigan, United States.

The vast outbreak of highly pathogenic avian influenza (HPAI) that occurred in the United States in 2015, resulted in the death of 49.6 million commercial birds. AVMA approved depopulation methods available, such as foam, containerized gassing, mechanical methods etc., can be problematic when depopulating commercial laying hen barns. Ventilation shutdown plus heat and/or CO₂ (VSD+) is approved only for emergency situations when no other method is available. VSD+ heat causes a decrease in humidity, and CO₂ can be a logistical and safety concern. Steam provides both heat and humidity, which limits hens' ability to reduce body heat through evaporative cooling or panting. Therefore, the objective of this experiment was to evaluate the effectiveness of VSD with steam as an alternative "plus" for depopulation of laying hens in a cage free system. Approximately 1,800 Lohmann Brown hens aged 56 weeks were reared in cage free aviaries (Big Dutchman Natura 60) in four rooms. Each room housed 450 hens and was further divided into three sections with 150 hens per section. Four VSD treatments were as follows: 1) control VSD+ heat to 41°C (VSD-H), 2) VSD with steam to 41°C (VSD-S), 3) VSD with heat to 41°C and then steam to maintain temperature (VSD-HS), and 4) VSD with steam to 41°C and then heat to maintain temperature (VSD-SH). Hens were monitored via cameras for time to first mortality and time to 100% mortality. Location of bird carcasses within each tier of the system (floor area, 1st tier, 2nd tier and nest boxes) was also observed. Data were analyzed in SPSS v. 28 and significance was at $P < 0.05$. Time to first mortality and last death for VSD-H, VSD-S, VSD-HS, and VSD-SH were 82.7 min and 180 min⁺; 56.6 min and 112.3 min; 49.6 min and 83.3 min; and 52 min and 103.6 min respectively. Hens in rooms with steam were significantly faster ($P < 0.0001$) in reaching time to first mortality and 100% mortality than the control VSD-H treatment. There was a greater ($P < 0.0001$) number of carcasses found in 1st tier than the floor area for VSD-HS treatment compared to VSD-SH and VSD-S treatments. Treatments that utilized steam were comparable in their ability to successfully depopulate laying hens based on the time to 100% mortality. The results indicate that steam alone or in combination with heat, can be used as an alternative "plus" for VSD+ depopulation of laying hens reared in a cage free system.

Key Words: aviary; depopulation; laying hens; steam; VSD

75 Reference Point Indentation: A novel method of assessing skeletal quality in poultry. Brittney Emmert¹*, Cara Robison², Thomas Siegmund¹, Gregory Fraley¹, Darrin Karcher¹, ¹Purdue University, West Lafayette, Indiana, United States, ²Michigan State University, East Lansing, Michigan, United States.

Noninvasive methods of assessing poultry bone biomechanics are lacking. Currently, the only relevant *in*

vivo imaging method is quantitative computed tomography (qCT), which is not easily accessible and involves transport of birds off-farm, introducing biosecurity risks. The ability to evaluate skeletal quality throughout a bird's life is needed to determine the impacts of treatments on bone over time. Reference point indentation (RPI) is a portable method of assessing bone biomechanical properties through a microindentation of the bone and has not been utilized in poultry. The technique has been successfully used *in vivo* in mammals, but *ex vivo* validation in poultry is needed. The objective was to validate RPI *ex vivo* by comparison to traditional methods using Pekin duck bones. Femurs, tibiae, and humeri from the left limbs were obtained from five market-age Pekin ducks. The fifteen bones were qCT scanned and analyzed for bone mineral density (BMD). Bone length was measured, and marks drawn at 25, 50, and 75% of the total length (proximal, midpoint, and distal location markers, respectively). For RPI assessment, bones were placed in a custom rig that prevented rotation. The BioDentTM RPI system was used on the anterior side of the bone at the three marked locations. Three measurements were taken at each location: at the drawn mark, and 2 mm above and below the mark. Data collected from RPI include indentation distance increase (IDI; difference in microindentation depth between the first and last loading cycle; indicator of bone resistance) and 1st cycle unloading slope (US; indicator of material stiffness). Humeri and tibiae were subjected to a 3-point bending test for fracture force at the midpoint of the bone. Data were analyzed using the COR function in R. All RPI measurement locations within each bone were averaged for correlations to BMD, and only RPI measurements from the midpoint were used for correlations to fracture force. When data for all bones are pooled together, IDI was inversely correlated with BMD ($r = -0.72, p = 0.002$), and US was correlated with BMD ($r = 0.62, p = 0.01$). Fracture force tended to be correlated with IDI ($r = 0.59, p = 0.07$) and inversely correlated with US ($r = -0.63, p = 0.05$). Generally, lower IDI and higher US values are preferred and indicate stronger bone. The negative correlation between IDI and BMD, and the positive correlation between US and BMD demonstrate that RPI can obtain results comparable to qCT. The RPI technique shows promise in evaluating poultry skeletal quality and provides a quicker, less laborious approach than qCT. The finding that RPI results do not appear to be related with fracture force requires further investigation.

Key Words: bone; breaking strength; computed tomography; Pekin duck; reference point indentation

76 Assessment of late-stage thermal manipulation on broiler chicken embryonic mortality and hatchability. Jorge Banegas¹*, Brittany Wall¹, Martha Rueda Lastres¹, Jeremiah Davis¹, Joseph Purswell², Charles Starkey¹, Jessica Starkey¹, ¹Auburn University, Auburn, Alabama, United States, ²Mississippi State, Starkville, Mississippi, United States.

Incubation temperature is one of the most important factors determining chicken embryo development and hatchability. Interest in the impact of thermal manipulation (TM) during critical periods of broiler embryogenesis has been renewed. Timing and duration of TM can alter egg moisture loss, embryonic mortality, hatchability, and chick weight (BW). The objective was to evaluate the effect of TM during the late-stage incubation on hatch characteristics in high-yielding broilers. Ross 708 × Yield Plus broiler breeder eggs (4 90-egg trays per incubator; total n = 2,160) ranging from 60 to 65 g were pre-warmed at 24 °C for 8 h and incubated at 37.5 °C and 65% RH from embryonic day (ED) 0 to 11. From ED 11 to 18, eggs were incubated at 1 of 3 temperatures: 37.5 °C (CTL), 36.4 °C (COLD), or 38.6 °C (HOT; n = 2 incubators per TM treatment). On ED 18, all eggs were individually weighed to determine moisture loss and transferred to baskets in hatchers set to 36.7 °C with 60% RH. After 514 h of incubation, all chicks from all hatchers were removed simultaneously, vent sexed, and weighed. Embryonic mortality (early, middle, late, and total dead; pipped; malpositioned) was assessed on all unhatched eggs 6 h after chick processing. Data were analyzed as a 1-way (TM) or 2-way (TM × sex) ANOVA with the GLIMMIX procedure of SAS (v9.4). Means were separated with the PDIF option at $P \leq 0.05$. Tendencies were declared when $0.0501 \leq P \leq 0.10$. Pre-incubation egg weights were similar among all treatments ($P = 0.5037$). Moisture loss tended to be greater in eggs from HOT incubators (8.4%) compared with those from COLD incubators (7.6%) but were similar to those incubated at CTL temperatures (8.3%; $P = 0.0793$). No differences among hatch of fertile, early, middle, and total dead, pipped, or malpositioned chicks as well as proportions of male and female chicks were observed among treatments ($P \geq 0.1463$). A 1.1 °C temperature gradient during late-stage incubation (from ED 11 to 18) tended to increase late-stage embryonic mortality in both COLD and HOT incubators compared with those at CTL temperatures ($P = 0.0932$). No TM × sex interaction was observed for chick weight at hatch, yolk-free chick weight, or residual yolk weight ($P \geq 0.0566$). Yolk-free chick weights and residual yolk weights from COLD incubators were heavier than those from HOT and CTL ($P \leq 0.001$). Chick hatch weight decreased as late-stage incubation temperature increased ($P \leq 0.001$) and is likely due to differences in hatch pattern among TM treatments. Late-stage incubation (ED 11 to 18) temperature gradients as little as 1.1 °C can alter late-stage embryonic mortality and chick weights at hatch underscoring the importance of careful hatchery management.

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

77 Evaluation of thermal variation during early-stage incubation on broiler chicken growth performance, carcass characteristics, and the breast meat quality defects, Wooden Breast and White

Striping. Brittany Wall^{1*}, Martha Rueda Lastres¹, Jeremiah Davis¹, Joseph Purswell², Charles Starkey¹, Jessica Starkey¹, ¹Auburn University, Auburn, Alabama, United States, ²United States Department of Agriculture, Mississippi State, Mississippi, United States.

Temperature changes during early-stages of incubation have shown to have contradicting effects on chick quality and post-hatch performance. The objective was to evaluate effects of temperature differences during early-stage incubation on broiler performance, carcass characteristics, and the incidence and severity of 2 meat quality defects. Ross 708 × Yield Plus broiler breeder eggs (n = 2,160) were incubated at 37.5 °C from embryonic day (ED) 0 to ED 3. On ED 4, COLD incubator setpoints were decreased to 36.4 °C, HOT incubator setpoints increased to 38.6 °C, and CTL incubators remained 37.5 °C (n = 2 incubators per treatment). On ED 11, HOT and COLD incubators were set to 37.5 °C until ED 18 when eggs were transferred to hatchers. Chicks were blocked by incubation treatment and sex (n = 6 replicates per treatment), reared in floor pens (30 birds per pen), fed a common diet in 3 phases (starter d 0 to 9, grower d 10 to 23, and finisher d 23 to 32), and processed at d 34. Incidence and severity of Wooden Breast (WB) and White Striping (WS) were determined 24 h post-processing by manual palpation. Data were analyzed as a 2-way ANOVA using SAS PROC GLIMMIX with incubation temperature and sex as main effects. The PDIF option was used for mean separation at $P \leq 0.05$ and tendencies were declared at $P \leq 0.10$. No incubation temperature × sex interactions were observed. While chicks from COLD incubators were heavier at placement ($P < 0.0001$), they were lightest on d 9, 23, and 32 post-hatch. Greater overall BWG was observed in HOT and CTL birds compared with those from COLD incubators ($P = 0.0285$). Birds from CTL incubators had greatest overall feed intake (FI; $P = 0.0084$). Male birds had increased BWG and FI ($P < 0.0001$), lower FCR during the grower phase ($P = 0.0109$), greater carcass part yields ($P < 0.0001$), and greater incidence of WS and WB severity compared with females ($P \leq 0.012$). Birds from the COLD incubators tended to have a 25-g reduction in breast weight (412 vs. 438 and 443 g; $P = 0.0656$) which resulted in a 6% decrease in breast meat yield compared with those from CTL and HOT incubators ($P = 0.004$). Thigh weight also tended to be negatively impacted by the early-stage COLD incubation treatment compared with HOT and CTL ($P = 0.0848$), whereas drum weight ($P = 0.0049$) and yield ($P = 0.0363$) were reduced in birds incubated with both the COLD and HOT treatments compared with CTL. Altering incubation temperature during early-stage incubation (ED 4 to 11) did not impact WB or WS incidence or severity ($P > 0.3762$). Overall, these results underscore the importance of careful incubation temperature management during early-stage incubation to optimize broiler growth performance and carcass yields.

Key Words: Incubation; Thermal manipulation; Broiler chicken; Growth performance; Wooden Breast

78 Effect of translucency and eggshell color on broiler egg hatchability and hatch chick weight. Leticia Orellana Galindo^{1*}, Duarte Neves⁴, James Krehling¹, Raquel Burin⁴, Patricia Carvalho^{2,1}, Leopoldo Almeida^{3,1}, Andrea Urrutia¹, Luis Munoz¹, Cesar Escobar Lobo¹, Matthew Bailey¹, Bernardo Chaves-Cordoba¹, Marco Rebollo⁴, Kenneth Macklin¹, ¹*Auburn University, Auburn, Alabama, United States*, ²*Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil*, ³*UFPR, Curitiba, Paraná, Brazil*, ⁴*Zimpro Corp, Eden Prairie, Minnesota, United States*.

A successful hatch has a considerable economic impact for all poultry companies. Previously several eggshell quality parameters such as eggshell weight, thickness, strength, and density have been demonstrated to impact hatchability. The aim of the current study is to describe the possible effects of eggshell translucency (1-3) and color (dark and light) parameters on hatchability and hatch chick weight. A total of 4320 eggs from Ross 708 breeder hens between 50 and 55 weeks of age were used for this study, where 1080 eggs were selected for these two parameters each day for 4 consecutive days (different flock each day). Eggshell translucency, color, and thickness were assessed during the egg's enrollment, while hatchability and chick weight were measured on day 21. For the evaluation of eggshell translucency, a 3-point subjective scoring system was used as the main categorical parameter based on the amount, size, and coverage of spot areas or mottling in the eggshell (1 = low, 2 = medium, 3 = high). Eggshell color from dark to light was evaluated using an electronic colorimeter. For egg thickness a noninvasive ultrasound gauge was employed. Data for this experiment was analyzed using the GLIMMIX procedure of SAS (V 9.4) and Tukey's HSD test was performed to separate means. Significant difference was considered between the means when $P \leq 0.05$. Results suggest that the color of eggshell was related to initial egg weight (**IW**) ($P = 0.0056$) on the day of collection and egg weight (**FW**) ($P = 0.0211$) on day 18 of incubation, in both cases dark eggs were heavier (**IW** = 67.05 g, **FW** = 60.72 g) than light eggs (**IW** = 66.49 g, **FW** = 60.09 g). Dark eggs had ~3.75% more eggs hatch from those set ($P = 0.0481$) and had a greater thickness ($P = 0.0019$) (417.47 μm) compared to light eggs (411.72 μm). Regarding translucency, final egg weight ($P = 0.0358$) was observed to be higher with score 1 eggs (60.74 g) compared to those with a score of 3 (59.92 g). Translucency score of 1 also had ~6.91% higher hatchability of eggs set ($P = 0.0127$) and greater chick weight ($P = 0.0385$) (45.79 g) in comparison to eggs with a translucency score of 3 (45.10 g). However, eggshell thickness was thinner ($P < 0.0001$) in eggs that had a translucency score of 1 (393.77 μm) than eggs that had a score of 2 (422.41 μm) and score of 3 (427.60 μm). An interaction ($P = 0.0292$) was observed for eggshell thickness, color, and translucency, where the eggs classified

as light and translucency of 1 had a thinner eggshell compared to those that were dark and had a translucency score of 3. It was concluded that dark colored eggs with a translucency score of 1 had the best effects on hatchability and chick weight.

Key Words: hatchability; eggshell; color; chick-weight; thickness

79 Evaluating the effect of day length (24, 20, 18 hours) during brooding on broiler performance. Garret Ashabranner^{1*}, Michael Czarick¹, Brian Fairchild¹, ¹*University of Georgia, Athens, Georgia, United States*.

In an effort to maximize early chick performance, broilers are typically grown with minimal to no dark period during the first week of growout. This is in stark contrast to previous research that found providing a dark period for older birds is beneficial in terms of broiler performance, health, and welfare. The objective of the study was to determine if providing chicks with a four or six-hour dark period during brooding would adversely affect performance. Four pens of 23 by-product male chicks were placed in six identical rooms (three rooms per treatment and control). Study 1 consisted of three 14d trials where treatment chicks were provided a four-hour dark period while control was given 24 hours of light for Days 0-7. Study 2 consisted of two 21d trials where treatment chicks were provided six hours of darkness while control was given 24 hours of light. In both studies, all birds were given four or six hours of darkness after Day 7. Birds were weighed and feed consumption was calculated on Days 3, 7, 10, and 14 for Study 1. Where in the second study bird weight and feed consumption was calculated on Days 3, 7, 10, 14, and 21. All data were analyzed using ANOVA and differences were considered significant at ($p < 0.05$). In Study 1, bodyweight, gain, and feed consumption of the control was significantly higher than the treatment ($p < 0.05$) for Days 3 and 7. However, bodyweight, gain, and feed consumption were higher in the treatment group on Day 10. In addition, the treatment group had higher feed consumption on Day 14. In Study 2, the control body weight, gain, and feed consumption were significantly higher ($p < 0.05$) for Days 3 and 7. No other significant differences were found in the second study. Although the control group birds had greater bodyweights on Days 3 and 7, body weights were similar after all birds were given the same day length in both studies. Feed conversion was not affected by the four or six-hour dark period. The results of these studies suggest that giving broiler chicks a dark period for the first seven days of a flock does not affect performance at 14 or 21 days of age. Additional studies will be conducted to evaluate the dark period impact on broiler performance and welfare closer to market age.

Key Words: growth; Chick management; lighting program; dark period; bodyweight

80 Effect of physical feed form on crop fill and growth performance of 3-d-old broiler chickens. Jorge Sandoval^{1*}, Brittany Wall¹, Jorge Banegas¹, Jessica Starkey¹, Charles Starkey¹, ¹*Auburn University, Auburn, Alabama, United States.*

Broiler growth and development depends on the optimal feed intake (**FI**) throughout the growing period. Optimal FI depends on several factors, including physical feed form which can be considered one of the most important factors affecting broiler growth rate. Broiler diets can be provided in 3 major forms: meal (**M**; finely ground and mixed ingredients), pellets (**P**; conversion of mash into hard-dry form through mechanical pressing), and crumbles (**C**; reground pellets into smaller particles). Assessment of crop fill at key times after placement can be beneficial for determining early brooding success. If adequate crop fill is not achieved, early growth rate can be compromised. The objective was to determine the effect of different feed forms on crop fill, crop diameter, and growth performance of broilers 3 d post-placement. On d of hatch, chicks (n = 480, Ross 708 x Yield Plus) were assigned to 24 pens by sex (20 birds per pen; 12 pens per sex) and fed 1 of 3 feed forms (M, P, and C) for 3 d. All dietary forms were of identical composition and were formulated to simulate commercial starter diets and meet bird nutrient requirements. Pen BW and FI were recorded at d 0 and 3 for performance calculations and crop fill assessments were done in all birds at 24 and 48 h post-placement. Data were analyzed as a 2-way ANOVA using SAS 9.4 PROC GLIMMIX and least square means were separated at $P \leq 0.05$. No feed form \times sex interaction was observed for any variable ($P \geq 0.1648$); therefore, only main effects are discussed. No differences were observed among treatments for crop fill at 24 and 48 h post-placement ($P > 0.1343$). Birds fed C diets had greater crop diameter compared with those fed P and M diets at 48 h post-placement ($P = 0.0284$). Female broilers had larger 48-h-crop diameter than male birds ($P = 0.0474$). On d 0, chick BW was similar among all treatments ($P > 0.5646$). On d 3, BW was higher on birds fed C diets and decreased in birds fed P and M diets ($P < 0.0001$). From d 0 to 3, C-fed birds had greater BWG and daily BWG than birds fed P and M diets ($P < 0.0001$). FI was greater in P-fed birds than those fed C and M diets ($P = 0.0026$). From d 0 to 3, female birds had higher FI than male birds ($P = 0.0348$). Birds fed C diets had lower FCR than those fed other feed forms ($P = 0.0002$). As expected, male birds had lower FCR than female birds ($P = 0.0073$). In conclusion, early growth performance and crop diameter were affected by both sex and physical feed form provided at placement. Male chicks had lower FI and FCR compared with females. Overall, providing chicks with starter feed in C form increased crop diameter and improved growth performance compared with those offered P and M diets.

Key Words: broiler; feed; growth performance; diet form; crop fill

81 Evaluation of novel litter amendments for use in the commercial broiler industry. Jorge Urrutia^{1*}, John Linhoss³, Anuraj Sukumaran¹, Hudson Thames¹, Jessica Wells¹, Tom Tabler², Kelley Wamsley¹, ¹*Mississippi State University, Mississippi State, Mississippi, United States*, ²*The University of Tennessee, Spring Hill, Tennessee, United States*, ³*Auburn University, Auburn, Alabama, United States.*

Acidifying poultry litter amendments (LA) are commonly used to improve litter quality via reducing litter pH, bacterial growth, and ammonia. However, pH control provided by acidifying LA typically lasts <two weeks and treatments can be harmful to equipment. The objective of this study was to evaluate the efficacy of inclusion rates (low, medium, high, and very high) of novel LA on various litter parameters of used commercial broiler litter (~13 years old, 50 flocks raised). The LA used in the study included: alum mud (AM; alum, bauxite, and sulfuric acid); biochar (BC, pine); superabsorbent polymers (SAP). Amendments were either mixed (MIX) in the litter or top dressed (TD) for all inclusion rates. BC and SAP LA were mixed into the litter, while AM was surface applied. An additional high inclusion treatment was added for each LA to compare the original application method (i.e., MIX or TD) to the opposite method. To compare LA to conventional methods, poultry litter treatment was surface applied at an industry standard rate (PLT-TD) and a control consisted of used broiler litter with no amendment applied (CON). Each treatment combination (n=17) was replicated eight times (n=136 total samples). LA were randomly applied as a randomized complete block design to litter contained within plastic boxes (experimental unit; 61 x 43 x 17 cm), depth of the litter was 8.9 cm. Boxes were stored within a controlled environment (35°C) for the experimental period. Water activity, litter moisture (LM), pH, and bacterial load (BL) for each treatment combination were determined at 0, 3, 7, 14, and 21 d. Data were analyzed using repeated measures over sampling d. Application of BC-MIX-VeryHigh had the highest water activity, while SAP-MIX-High had the lowest ($P=0.0004$); all other treatments were similar, including the CON and PLT. SAP-TD-High had the highest LM, higher than all other treatments except SAP-MIX-VeryHigh; AM-TD had the lowest LM, though similar to PLT and CON, as well as all BC tested ($P<0.0001$). Application of BC-TD-High had the highest BL, but only higher than SAP-MIX-VeryHigh and SAP-TD-High ($P=0.0017$). The highest pH was found with application of BC-MIX-VeryHigh, though similar to CON, other BC treatments, and AM-TD-Low and AM-MIX-High ($P=0.001$). Increasing SAP above Medium inclusion reduced pH similar to that of PLT; the lowest pH was measured with SAP-MIX-High. These results suggest that depending upon SAP application method and inclusion, resulting metrics may be similar (i.e., water activity and BL) or better (i.e., pH) to that observed with PLT. However, future research is necessary to refine these LA treatments to determine their efficacy with live birds prior to testing in a commercial setting.

Key Words: water activity; litter moisture; litter amendments

82 The effect of nicarbazin on broiler body temperature when exposed to increases in environmental temperature and RH. Kristin Miles^{1*}, Michael Czarick¹, Brian Fairchild¹, ¹*University of Georgia, Athens, Georgia, United States.*

Nicarbazin has been reported to increase bird body temperature and impair the bird's ability to cope with heat stress. The objective of this study was to determine the effect of nicarbazin on bird body temperature during times of elevated environmental temperatures and RH levels. Body temperatures of 80 Cobb500 off sex male chicks were recorded using a RFID LifeChip® that was surgically placed subcutaneously on the left flank post hatch and eight six-inch antennas connected to a T-Chip®Chick reading system. Chicks were randomly assigned to two treatments, nicarbazin diet and control diet, on Day 8. Nicarbazin was fed to the treatment birds from Days 8 to 17. After Day 17, all birds were fed the control diet. Birds were reared under the breeder's recommended temperature curve starting at 34C on Day 0 and gradually decreasing to 24C on Day 23. Environmental temperature was increased by 2.8C for 6 hours on Days 9,11,16, and 20. RH was increased to 75-80% for 7 hours on Days 13 and 14. Body temperature data were analyzed by period of environmental change using a generalized linear model and significance was determined at $P<0.05$. During the first period of increased ambient temperature, the birds fed nicarbazin had a significantly higher body temperature (0.17C) than those fed the control diet ($P=0.0002$). Birds fed nicarbazin continued to have a significantly higher body temperature during the second period of increased ambient temperature ($P=0.0277$) but the difference in body temperature was not as large (0.09C). There was not a significant difference in body temperature between the treatment groups during the third period of elevated environmental temperature ($P=0.3126$), suggesting birds fed nicarbazin acclimated to the rapid change in ambient temperature. When the ambient temperature was returned to 26.6C after the third period of heat stress, the nicarbazin fed birds had a significantly lower body temperature than those fed the control diet ($P<0.05$). This difference continued for four days through the time of nicarbazin removal from the treatment diet until the beginning of the fourth period of elevated ambient temperature. Both treatment groups were fed control diet during this period where there was not a significant difference in body temperature between the two treatment groups ($P>0.05$). The rapid change in RH did not cause a significant difference in body temperatures of birds in either treatment ($P>0.05$). Nicarbazin does not cause an increase in body temperature during thermoneutral temperatures but causes birds to have a larger body temperature increases during periods of elevated ambient temperature.

Key Words: heat stress; anticoccidial; relative humidity

83 Effect of probiotics (Galliprotect WS, Bacillus Licheniformis) on the gut health of broiler chickens experimentally induced from dysbacteriosis. Zeeshan Haider^{1*}, ¹*University of Veterinary and Animal Sciences, Lahore, Faisalabad, Pakistan.*

One hundred twenty commercial day-old chicks of broiler were procured from local market and reared in controlled house. The chicks were divided in four groups i.e. A, B, C and D with 30 birds in each group. Recommended vaccination schedule by National Disease Control Committee, Pakistan Poultry Association followed for broiler chicks. As birds arrived, 5 chicks were randomly selected to check the either E.coli present or not. Feed and water as ad libitum. Cultured E.col was used as a challenge. E.coli was cultured in macconkey agar and post- incubation, bacterial cells collected, reconstituted in saline, quantified by total viable count and diluted. On the 7th day, one ml of cultured avian pathogenic strain of e.coli was given to 60 chickens orally. On the fifth day, ND killed and H9 vaccines were injected as sub cutaneously. B1 strain of ND was given also by eye drop. On the 11th day, gumboro intermediate plus type was given in water against infectious bursal disease, bioveta. From 18th day to onwards, the infected birds were treated with probiotic, three different types of antibiotics and dutrion, chlorine dioxide. Probiotic was given to group C infected birds and group D birds were further divided into 3 sub-groups. On the 30th day, five birds from group B, five from group C and 2 birds from each D1, D2 and D3 were checked to know the severity of e.coli and recovery of birds from infection using different products. Gut health scoring parameters, Ballooning of gut, Tonus of intestine, Thickness of GIT, Redness of mucosal surfaces Edges of intestines Intestinal contents Inflammation Goblet cell account Absorption area of intestine Immune organ status and weight gain and FCR. The used of probiotics increased the immunity of birds by maintaining balanced gut microflora and increased the lengths, thickness of gut, reduced the inflammatory chemical mediators improving feed intake and digestion, neutralizing enterotoxin and stimulating the immune system & by producing antimicrobial peptides, and improved the weight gain and feed conversion ratio in broiler. Further studies can be done to know the effects of probiotics on the production of ammonia and level immunoglobins in the gut of the chickens and we need to use probiotics to produce antibiotics free meat in market. We need to give awareness on the use of probiotics rather than the use of antibiotics in broiler production.

Key Words: Probiotics; Gut health; Antibiotics; Management; Gut micro-flora

Student Competition: Metabolism and Nutrition, General Nutrition

84 Evaluation of methodology to determine energy balance of corn and soybean meal for broilers. Stephanie Philpot^{1*}, Aaron Cowieson², William Dozier, III¹, ¹*Auburn University, Auburn, Alabama, United States*, ²*DSM Nutritional Products, Kaiseraugst, Switzerland*.

Accurate energy values for diet formulation help meet broiler performance and economic objectives. Previous research has shown that energy balance of test ingredients may be affected by assay methodology. Two experiments were conducted to evaluate three assay methodologies to determine digestible (DE) and metabolizable (ME) energy of corn and soybean meal. Experiment 1 evaluated corn and consisted of six dietary treatments, including a semi-purified diet, a direct diet (94% corn meal), two basal diets, and two diets where corn was substituted at either 15 or 30% into respective basal diets. Experiment 2 evaluated soybean meal and consisted of six dietary treatments, including a semi-purified diet, a direct diet (43% soybean meal + 52% dextrose), two basal diets, and two diets where soybean meal was substituted at either 10 or 20% into respective basal diets. Basal diets in both experiments were formulated to provide 1.15% digestible Lys in the substitution diets. The semi-purified diet was included to estimate endogenous losses of energy. All diets contained titanium dioxide as an indigestible marker. In both experiments, day-old YP × Ross 708 male chicks were placed into battery cages (9 chicks/cage) and were provided a common starter diet from 1 to 17 D of age. At 18 D of age, birds were provided with test diets. Excreta was collected at 21 and 22 D of age and feed was weighed at 22 D of age. Terminal ileal digesta was collected at 23 and 24 D of age. Titanium dioxide concentration, N, and gross energy were determined in excreta and ileal digesta samples. Each experiment was conducted as a randomized complete block design with 16 replicates per diet. Apparent DE and ME, standardized DE and ME, and N-corrected apparent ME were determined for both corn and soybean meal using the direct method and the substitution method at two inclusions. Energy utilization was analyzed using analysis of variance. In Experiment 1, determined apparent and standardized ME of corn were reduced utilizing the direct method compared with substitution at either 15 or 30% ($P < 0.0001$; 3,290, 3,635, or 3,636 kcal/kg AME, respectively). In Experiment 2, determined apparent and standardized ME and N-corrected ME of soybean meal were reduced in the direct method compared with substitution at 10 or 20% ($P \leq 0.0024$; 2,211, 2,496, or 2,437 kcal/kg AME, respectively). In both experiments, standard error was increased using the substitution method compared with the direct method due to attributing all variance in dietary energy to the test ingredient. These experiments indicated that the direct method may underestimate energy of test ingredients, while the substitution method may increase variance of the determined energy.

Key Words: Energy; Broiler; Assay; Endogenous Losses; Ingredient

85 Poultry meal autoclaving time-related reduction in energy concentrations for broiler chickens. Jung Yeol Sung^{1*}, Markus Wiltafsky-Martin², J. Caroline González-Vega², Olayiwola Adeola¹, ¹*Purdue University, West Lafayette, Indiana, United States*, ²*Evonik Operations GmbH, Hanau, Germany*.

The objective of this study was to determine effect of autoclaving on energy concentrations in poultry meal fed to broiler chickens. Poultry meal from the same batch was autoclaved at 134°C for 0, 30, 60, 90, 120, 150, or 180 min for 7 samples. Eight experimental diets consisting of a basal diet based on corn and soybean meal and 7 test diets replacing 15% of energy-contributing ingredients in the basal diet with the 7 poultry meal samples were used. A total of 512 male broiler chickens (Cobb 500; initial body weight = 660 ± 80 g) at d 17 post hatching were allotted to 8 dietary treatments in a randomized complete block design with body weight as a blocking factor. Each dietary treatment contained 8 replicate cages with 8 birds per cage. Birds had a free access to the experimental diets and water during the experimental period. Approximately 100 g of excreta samples were collected daily from d 20 to 22 at 0800 h. On d 22, birds were euthanized by CO₂ asphyxiation, individually weighed, and dissected for the collection of ileal digesta. Samples of ileal digesta were collected from the distal two-third portion of the ileum (i.e., from Meckel's diverticulum to approximately 20 mm proximal to the ileocecal junction) by flushing with distilled water. Data were analyzed using the MIXED procedure of SAS. The model included diet as a fixed variable and block as a random variable. Orthogonal polynomial contrasts were used to determine the effect of autoclaving time. Experimental unit was a cage, and significance and tendency were declared at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively. Ileal digestible energy (from 4,257 to 3,868 kcal/kg DM) and metabolizable energy (from 3,591 to 3,306 kcal/kg DM) in poultry meal tended to linearly decrease ($P = 0.055$ and 0.063 , respectively) as autoclaving time increased. However, effect of autoclaving time on nitrogen-corrected metabolizable energy in poultry meal (from 3,579 to 3,393 kcal/kg DM) was not observed. In conclusion, energy utilization response to heat damage should be considered when using metabolizable energy of poultry meal in feed formulation for broiler chickens.

Key Words: broiler; energy; feed formulation; autoclaving; poultry meal

86 Validation of the 3-point model for the determination of metabolizable energy using the regression method in broiler chickens. Tanner Wise^{1*},

Olayiwola Adeola¹, ¹*Purdue University, West Lafayette, Indiana, United States.*

The objective of the following studies was to investigate the validity of the use of 3 points in determining metabolizable energy (ME) using the regression method in broiler chickens. To test this, 2 experiments were conducted: experiment (Exp) 1 using canola meal (CM; protein substrate) as the test ingredient (TI), and Exp 2 using wheat grain (energy substrate) as the TI. Each Exp utilized a 4-diet model with a corn-soybean meal-based reference diet and 3 test diets with increasing concentrations of the respective TI. Test diets (TD) contained 100, 200, or 300 g/kg CM, added at the proportional expense of all energy contributing ingredients for Exp 1, and 150, 300, or 450 g/kg wheat for Exp 2. For each Exp, 192 Cobb 500 male birds were weighed and allotted to 1 of the 4 treatments at d 21 post-hatching in a randomized complete block design, with BW as the blocking factor. Birds were fed diets for 5 d and excreta was collected the final 3 d. Growth performance and metabolizability responses were evaluated for linear and quadratic effects using orthogonal contrasts, and ME of the TI was determined by regressing the TI-associated ME against the dry matter intake of the test ingredients using a generalized linear model. For each Exp, 4 data sets were used to determine ME, set 1 contained data from all 4 diets while each of the other 3 excluded 1 of the TD. For Exp 1, dietary concentration of CM had no effect on growth performance. For Exp 2, increased dietary wheat concentrations linearly decreased ($P < 0.01$) BW gain and feed efficiency. For both Exp, increases in dietary inclusion of the TI elicited linear decreases ($P < 0.01$) in the metabolizability of DM and GE as well as decreasing the ME of the TD. The ME of CM obtained from the 4 data sets ranged from 1731 to 1992 kcal/kg, however, the data set that excluded the highest concentration of the TI produced the estimate of 1992 kcal/kg, but the other three sets ranged only from 1731 to 1793 kcal/kg. In addition to the increased estimate from the data set excluding the high CM, the standard error of the estimate was 42% higher than that of the mean of the other 3 sets (126 vs. 89 kcal/kg). The ME of wheat from the 4 data sets had a much smaller range of 3041 to 3106 kcal/kg, however, the standard error of the set excluding the highest wheat inclusion was 36% higher than the mean of the other 3 sets (131 vs. 96 kcal/kg). These data indicate that there is no numerical difference in the variations of estimates between the 3 and 4-point models, provided that the inclusion of the TI is adequate as both models represent the linearity and variability of responses.

Key Words: broiler chicken; metabolizable energy; wheat; canola meal; regression method

87 Metabolizable energy and amino acid digestibility of corn from different origin for male broilers. Jose Vargas^{1*}, Joseph Gulizia¹, Susan Bonilla¹, Santiago Sasia¹, Wilmer Pacheco¹, ¹*Auburn University, Auburn, Alabama, United States.*

Two experiments were conducted to determine the apparent metabolizable energy (AME) and amino acid (AA) digestibility of corn from USA, Argentina, and Brazil fed to male broilers. In experiments 1 and 2, a total of 432 and 324 d-old YPM x Ross 708 male broilers were randomly sorted in 48 and 36 battery cages, respectively, fed a common starter diet from 0 to 11 d of age, and subjected to dietary treatments from 12 to 18 d of age. Both experiments were designed in a randomized complete block design with 12 replications per treatment. In experiment 1, dietary treatments consisted of a grower control diet with 30% dextrose inclusion, and 3 test diets obtained by the substitution of dextrose with corn from each origin. Starting at 16 d of age, a 72-h AME assay using the total collection method was conducted. Feed disappearance and excreta weights were recorded daily to calculate energy intake and excretion. Additionally, a 250 g excreta sample free of feed and feathers was collected from each cage daily for gross energy analysis. In experiment 2, an apparent ileal AA digestibility assay using the index method was conducted. Dietary treatments consisted of 3 diets with corn from each origin being the only source of AA. Ileal digesta was collected from all the birds on d 18 to analyze AA digestibility. All the data were analyzed as a one-way ANOVA using the GLM procedure of JMP PRO 15. No significant differences ($P > 0.05$) were observed on AME on a dry matter basis among corn origin. A minimum AME variability of only 43 kcal/kg was observed between corn from BRA (3,694 kcal/kg) and corn from the US (3,651 kcal/kg), which had the highest and lowest AME, respectively. Similarly, no significant differences ($P > 0.05$) were observed on the digestibility of most studied AA. However, corn from BRA (77.45%) had a significantly higher ($P \leq 0.05$) tryptophan digestibility when compared to corn from USA (72.53%). Overall, a low variability of AME and AA digestibility was observed, which indicates a very similar nutrient utilization by the birds, regardless of corn origin.

Key Words: amino acid digestibility; apparent metabolizable energy; corn

88 Standardized amino acid digestibility and true metabolizable energy for several increased protein ethanol co-products produced using back-end fractionation systems. Benjamin Parsons^{1*}, Pam Utterback¹, Carl Parsons¹, Jason Emmert¹, ¹*University of Illinois at Urbana Champaign, Urbana, Illinois, United States.*

Precision-fed rooster trials were conducted to evaluate the standardized amino acid (AA) digestibility and TME_n of conventional distillers dried grains with solubles (DDGS), corn fermented protein (CFP), high protein-DDGS (HP-DDGS), and reduced fiber high protein-DDGS (RFHP-DDGS) produced using post-fermentation back-end fractionation systems. Eight samples of CFP collected from different ethanol plants over a three-year period, two samples of HP-DDGS, two samples of RFHP-DDGS, and

four samples of conventional DDGS were evaluated. The TME_n was determined using conventional White Leghorn roosters, while cecectomized roosters were used to determine standardized AA digestibility. Three to six roosters per treatment were fasted for 26 h prior to crop intubation with 27 g of sample, and excreta were collected for 48 h post feeding. Statistical analyses were conducted using a one-way ANOVA for a completely randomized design with treatment as a main effect, and pairwise treatment comparisons were conducted using the least significant difference test. The probability level for the ANOVA and pairwise treatment comparisons was $P < 0.05$. Eight samples of corn fermented protein were found to contain a mean of 56% CP (DM basis) compared with a mean of 32% for conventional DDGS1 and 2. The mean TME_n of CFP (3,556 kcal/kg DM) was higher ($P < 0.05$) than conventional DDGS1 and 2 (2,767 kcal/kg DM); mean standardized AA digestibility values for CFP were similar to conventional DDGS and ranged from 88% to 94%. The mean digestible Lys, Met+Cys, and Thr concentrations for conventional DDGS were 0.79%, 1.12%, and 0.94%, respectively, whereas those for CFP were 1.74%, 2.06%, and 1.88%, respectively (DM basis). Two samples of HP-DDGS contained a mean of 51% CP (DM basis), a mean TME_n of 3,325 kcal/kg DM, a mean standardized AA digestibility value of 89.5%, and mean concentrations of digestible Lys, Met+Cys, and Thr, which were 1.53%, 1.77%, and 1.60%, respectively (DM basis). The mean CP content of two RFHP-DDGS was 48% and the mean TME_n was 3,711 kcal/kg DM, which was higher ($P < 0.05$) than conventional DDGS3 and 4 (2,920 kcal/kg DM). Mean standardized AA digestibility value of RFHP-DDGS was 90% and digestible Lys, Met+Cys, and Thr concentrations were increased from 0.82%, 1.01%, and 0.95% for conventional DDGS, respectively, to 1.00%, 1.59%, and 1.45% for RFHP-DDGS, respectively (DM basis). The results indicate that these high protein corn ethanol co-products produced by back-end fractionation systems have increased nutritional value for poultry compared with conventional DDGS.

Key Words: amino acid digestibility; true metabolizable energy; distillers dried grains with solubles; corn fermented protein; increased protein corn co-products

89 Determination of TME_n , standardized amino acid digestibility, phosphorus digestibility, and phosphorus bioavailability of fermented soybean meal and fermented full-fat soybeans fed to chickens. Minoy Cristobal^{1*}, Benjamin Parsons², Pam Utterback¹, Hans Stein¹, Carl Parsons¹, ¹University of Illinois at Urbana Champaign, Urbana, Illinois, United States, ²University of Arkansas, Fayetteville, Arkansas, United States.

Four experiments were conducted to evaluate the nutritional value of dehulled solvent-extracted soybean meal (SBM-CV), fermented SBM-CV (F-SBM), dehulled full-fat soybeans (FFSB), and F-FFSB. The fermentation process

increased the protein content by approximately two percentage units and decreased the stachyose and raffinose content to near zero in the F-SBM and F-FFSB. In Experiments 1 and 2, two precision-fed rooster assays were performed to determine the nitrogen-corrected true metabolizable energy (TME_n) and standardized amino acid (AA) digestibility among the test ingredients using conventional and cecectomized roosters, respectively (6 roosters per treatment, fed 25 g). Dietary treatments were arranged in a 2×2 factorial to evaluate the effects of fermentation and SBM type, and the data were analyzed as a two-way ANOVA with alpha set at $P < 0.05$. There was an interaction ($P < 0.05$) between fermentation and SBM type for TME_n . Fermentation of SBM-CV increased TME_n , while fermentation of FFSB decreased TME_n . There was no main effect of fermentation or SBM type for indispensable AA digestibility values except for Lys, where a negative effect ($P < 0.05$) of fermentation was observed. In Experiment 3, an ad libitum-fed broiler chicken assay was conducted to determine apparent ileal P digestibility and total tract P retention at two Ca levels among the 4 test ingredients. Diets contained a Ca:non-phytate P ratio of either 2 or 7.5. Dietary treatments were arranged in a $2 \times 2 \times 2$ factorial with fermentation, SBM type, and Ca level as the main effects (5 pens of 5 chicks per diet). Data were analyzed as a three-way ANOVA with alpha set at $P < 0.05$. Higher ($P < 0.05$) apparent ileal P digestibility values were observed at the lower Ca level than at the higher Ca level. At the higher Ca level, fermentation increased ileal P digestibility and total tract P retention for both conventional and full-fat samples. Experiment 4 was conducted to determine relative P bioavailability (RBV) of the test ingredients compared with KH_2PO_4 using crossbred chicks (5 pens of 5 chicks per diet). Multiple regression of bone ash (mg/tibia and %) on supplemental P intake yielded slope-ratio RBV varying from 23% to 48%. Fermentation did not significantly affect RBV of P in SBM-CV, but increased ($P < 0.05$) RBV of FFSB. In summary, fermentation increased TME_n in SBM-CV but had a negative effect on Lys digestibility for both SBM-CV and FFSB, suggesting possible heat damage during the fermentation process. Fermentation had a positive effect on apparent ileal P digestibility and total tract P retention for both SBM-CV and FFSB when diets contained 0.75% Ca and also increased RBV of P in FFSB based on bone ash responses in chicks.

Key Words: Soybean meal; Phosphorus; Fermentation; Metabolizable energy; Amino acid digestibility

90 The impacts of amino acid density and phytase activity variability on D1-38 Ross 308 male broiler performance and processing yields. Brendan Liebross^{1*}, Courtney Poholsky², John Boney³, ¹The Pennsylvania State University, Manalapan, New Jersey, United States, ²Penn State University, Howard, Pennsylvania, United States, ³Penn State University, University Park, Pennsylvania, United States.

Nutrient segregation in commercial poultry houses has recently been documented. Nutrient variability throughout the house may contribute to differences in broiler performance and feed efficiency. Recent on-farm nutrient segregation literature reported amino acid (AA) variability of more than 10%, and phytase activity (PA) variability of more than 50% across defined regions of the feed line. Because broiler movement decreases with age, it is important to understand the performance and processing impacts of on-farm nutrient segregation. An experiment was designed to study the impacts of AA density and PA levels on d1-38 broiler performance and processing yields. Dietary treatments were arranged as a 2 AA density (95% or 105% of recommendations) x 3 PA (250 FTU/kg; 500 FTU/kg; 750 FTU/kg) factorial in a randomized complete block design. A total of 1,488 Ross 308 male broilers were placed in pens with 31 birds per pen and eight replicate pens per treatment. A common starter crumble was provided from d1-10 and dietary treatments were applied during the Grower (d11-d24) and Finisher (d25-d38) phases. Each diet differing in AA density was formulated to include PA at 500 FTU/kg of feed. However, phytase was not included during batching and the appropriate phytase dose (250, 500, or 750 FTU/kg) was applied to the pelleted feed at Penn State. Birds and remaining feed were weighed on days 10, 24, and 38. On d38, individual BW were obtained to calculate BW coefficient of variation (CV%). Additionally, three birds per pen, \pm 50-g of the average bird weight, were tagged and collected for processing yield determinations. Data were subjected to a two-way ANOVA using the GLM procedure of SAS with $P < 0.05$ being deemed significant. Main effects did not interact during the d1-38 performance period nor for d39 processing yields ($P > 0.05$). The main effect of AA density improved LWG by 115 g/bird ($P = 0.001$) and BW by 121 g/bird ($P = 0.001$) when AA density was increased to 105% of breed recommendations. Feed conversion ratio was improved by 0.070 when broilers were provided the diet with excess AA ($P < 0.001$). Neither the main effect of AA density nor PA affected feed intake or BW CV% ($P > 0.05$). The main effect of PA did not affect d1-38 LWG, BW, FCR, breast weight, or breast yield ($P > 0.05$). Broilers provided the diet with AA at 105% of breed recommendations increased breast weight by 55-g and breast yield by 3.3% compared to those provided diets with AA at 95% of breed recommendations. Within the parameters of this experiment, differences in AA density influenced performance and processing yields, suggesting that on-farm nutrient segregation influences flock performance.

Key Words: Phytase; Broiler Chickens; Amino Acid Density ; Nutrient Segregation

91 Effects of normal oleic extruded expeller and full-fat, and high oleic soybean meals on live performance, carcass and meat quality, and meat fatty acid composition of broilers. Muhammad Ali^{1*}, Maria Alfaro-Wisaquillo¹, Gustavo Quintana-Ospina¹, Michael Joseph¹,

Danny Patino¹, Ondulla Toomer², Thien Vu², Lisa Dean², Edgar Oviedo- Rondón¹, ¹*North Carolina State University, Raleigh, North Carolina, United States*, ²*USDA, SEA ARS, Raleigh, North Carolina, United States*.

High oleic soybean meal (SBM) may positively affect broiler meat quality. This experiment evaluated the effects of normal-oleic extruded expeller (NO-EE) and full-fat soybean meal (NO-FF), and high-oleic full-fat (HO-FF) soybean meal (SBM) on broiler live performance, carcass and cut-up parts yield, and meat fatty acid composition. These extruded SBM were produced in the same location under similar processing conditions. Nutrient and energy content of SBM were obtained by NIRS and wet chemistry. Diets were formulated to be isoenergetic, isonitrogenous, and had identical digestible Lys, TSAA, Thr, Val, Ca, and available P in the three feeding phases. Five hundred and forty Ross-708 male broilers were raised on floor pens, with 18 birds per pen and 10 replicates per treatment in a completely randomized design. Chickens were fed starter diets in crumbles up to 14 d of age, while grower (15 – 35 d) and finisher diets (36 – 47 d) were fed in pellets. Chickens were weighed at 7, 14, 35, and 47 d. At d 48, four broilers per pen were processed, and cut-up in parts. Breast samples were collected and evaluated for quality and fatty acid content. Broilers fed diets with NO-EE were heavier ($P < 0.05$) than chickens fed diets with full-fat SBM at d 7 (176 g), 14 (526 g), and 35 d (2,494 g), but no treatment effect ($P > 0.05$) was observed at 47 d of age. NO-EE had better FCR ($P < 0.05$) than HO-FF at 7 d and 47 d, while NO-FF had intermediate results. At d 35, broilers fed NO-FF had similar FCR than NO-EE and better than those fed HO-FF SBM. Carcass yield was also higher for broilers fed NO-EE than HO-FF, while NO-FF was intermediate. No effects of dietary treatments were detected ($P > 0.05$) on cut-up part yields. After 24h, breast pH was lower for HO-FF than NO-EE and NO-FF. Breast meat color, cooking, and dripping loss were not affected ($P > 0.05$) by treatments. Breast fillets without wooden breast (score 1) were higher ($P < 0.05$) for NO-FF than the other two treatments. There were no significant effects ($P > 0.05$) of SBM source on white stripping or spaghetti muscle. The breast meat fatty acid profile (g fatty acid/ 100 g of all fatty acids) was affected ($P < 0.001$) by the SBM source. Broilers fed diets containing HO-FF SBM had 54 to 86% more oleic acid in the breast muscles than NO-FF and NO-EE. In contrast, the linoleic acid was reduced by 42 to 57% in broilers fed HO-FF. Saturated fatty acids like palmitic and stearic had the lowest concentration in meat from HO-FF broilers. In conclusion, broilers fed HO-FF enriched oleic acid content of breast meat while reducing the saturated fatty acid content, but had lower live performance and carcass yield than NO-EE SBM. High oleic full-fat SBM may enhance the quality of broiler meat.

Key Words: broilers; fatty acids; performance; soybean meal; Breast meat

92 Effects of improvements to pellet quality on commercial hen and tom turkey performance and processing variables. Courtney Poholsky^{1*}, Morgan Watt¹, Logan Erb¹, Alyssa Lyons¹, John Boney¹, ¹*Penn State University, University Park, Pennsylvania, United States.*

Performance benefits associated with pellet quality (PQ) improvements are well documented for broiler chickens. It has been suggested that turkeys are more sensitive to PQ improvements than broilers, but PQ research using modern commercial turkeys is limited. Therefore, three experiments (EXP) were conducted to investigate the effects of PQ on commercial hen and tom turkey performance and processing variables. In all EXP, a poor pellet quality (PPQ) feed and improved pellet quality (IPQ) feed were provided to eight replicate pens of Nicholas Select turkeys. Hen turkeys were provided the PPQ and IPQ feed treatments from 5-16 weeks in EXP 1, and from 13-16 weeks in EXP 2. In EXP 3, tom turkeys were fed PPQ and IPQ feed from 5-19 weeks of age. During the experimental periods for each EXP, turkeys were placed on a commercial feeding program that followed Aviagen nutrient recommendations. The IPQ feed was manufactured at a commercial feed mill using techniques known to generate high quality pellets. Feed was augered into a bulk bin and then systematically split into two equal allotments (PPQ and IPQ). Four 22.68-kg feed bags were methodically selected and removed from each allotment for initial pellet-to-fine ratio (P:F) determination using a modified particle size separator fitted with a No. 5 sieve. To create PPQ treatments, calculated portions of pellets from the initial PPQ allotment were ground using a hammer mill, creating fines. Calculated amounts of feed and fines were then mixed to reduce the P:F by 30 percentage points for each phase. All performance and processing metrics were analyzed in a one-way ANOVA using the GLM procedure of SAS. In EXP 1, hen performance was not influenced by PQ from weeks 5-12 ($P>0.05$). However, hens fed IPQ feed from weeks 13-16 had improved FCR by 0.237 compared to those fed PPQ feed ($P=0.012$). This performance improvement in the final four weeks of heavy hen production contributed to a 0.107 FCR improvement from weeks 5-16 when providing IPQ feed ($P=0.037$). In EXP 2, hens consuming IPQ feed had improved FCR by 0.242 compared to those fed PPQ feed ($P=0.010$), offering a potential PQ improvement strategy to feed mill operators. In EXP 3, improvements in LWG and BW were observed in toms consuming IPQ feed from 5-14 weeks of age ($P<0.05$). However, PQ did not affect tom performance when considering weeks 5-19 ($P>0.05$). Processing yields were not influenced by PQ in any EXP ($P>0.05$). Overall, improvements in turkey performance can be achieved by implementing an appropriate PQ improvement strategy.

Key Words: feed manufacture; nutrition; turkeys; pellet quality

93 Assessing retention time during the conditioning process of a starter diet fed from day 1 to 21 and the

impact on broiler performance. Abigail McConnell^{1*}, Rachel Strobeck¹, Joseph Gulizia², Wilmer Pacheco², Kevin Downs¹, ¹*Middle Tennessee State University, Murfreesboro, Tennessee, United States,* ²*Auburn University, Auburn, Alabama, United States.*

The purpose of this research was to assess the effect of three retention times during conditioning on broiler performance, nutrient digestibility, and apparent metabolizable energy (AME) from 1 to 21 d. A total of 288 Cobb 500 byproduct males were distributed randomly into 18 battery cages, with 6 replicate cages per treatment and 16 birds per cage. A corn-soybean meal-based diet was mixed using a twin shaft mixer. After mixing, diets were steam conditioned at 82°C with three retention times (40, 80, and 120 seconds), pelleted through a 4.0-mm pellet die, cooled using a counterflow pellet cooler, and crumbled. Body weight and feed consumption data was collected at 7-d intervals. Feed conversion was corrected for mortality. Replicate fecal samples were collected from each cage on d 20 and 21 for AME determination. At the end of the study, 5 birds per cage (30 birds per treatment) were euthanized and ileal digesta collected. Data were analyzed as a completely randomized design, with battery cage representing the experimental unit, using the GLM procedure of SAS to assess treatment main effects. Means were separated using Tukey's HSD and statistical significance considered at $P \leq 0.05$. Likewise, linear regression analysis was conducted using the REG procedure of SAS. Overall, there were no significant treatment main effects for d 1 to 7 ($P = 0.996$), 1 to 14 ($P = 0.997$), or 1 to 21 ($P = 0.565$) feed consumption; or d 1 to 7 ($P = 0.961$), 1 to 14 ($P = 0.292$), or 1 to 21 ($P = 0.158$) FCR. Although there were no significant treatment effects for BW on d 7 ($P = 0.691$), 14 ($P = 0.087$), or 21 ($P = 0.087$), there was a d 14 and 21 BW tendency ($0.05 < P < 0.10$). Broilers fed the diet with 40 seconds retention time during conditioning had 46 grams higher BW (1,118 vs. 1,072 grams) and 4-points lower FCR (1.21 vs. 1.25 g:g) compared to broilers fed diets with 120 seconds retention time. This was, in part, confirmed by linear regression showing a significant inverse relationship between conditioning process time and BW at d 14 ($P = 0.029$) and approaching significance at d 21 ($P = 0.080$). Apparent ileal nutrient digestibility and AME data is still being collected and not presented here. Overall, there is some indication that extended retention times during conditioning may have negative influences on BW and FCR. This should be further assessed for a longer growing period and on a larger scale.

Key Words: Broilers; Performance; Conditioning; Retention Time

94 Grinding during pelleting as influenced by corn particle size and pellet diameter. Susan Bonilla^{1*}, Joseph Gulizia¹, Jose Vargas¹, Santiago Sasía¹, Leopoldo Almeida¹, Patricia Soster¹, Wilmer Pacheco¹, ¹*Auburn University, Auburn, Alabama, United States.*

Particle agglomeration during pelleting improves feed handling, reduces wastage, increases intake, and improves broiler performance. However, the additional grinding that occurs during pelleting is usually ignored or difficult to measure, leading to inconclusive results regarding optimum particle size in broiler diets. The aim of this study was to determine the degree of grinding that occurs during pelleting by determining the internal particle size in the microstructure of the pellet based on the initial corn particle size as well as the diameter of the pellet. Corn was ground using a two-pair roller mill to obtain four particle sizes (855, 1659, 2043 and 2534 μm). Ingredients were blended using a twin shaft mixer to produce the mash diets. After mixing, mash samples produced from each corn particle size were collected for particle size analysis. Thereafter, mash diets were steam conditioned at 77°C with a retention time of 45 seconds and pelleted using two pellet dies (3.0 and 4.0-mm). Pelleted samples were collected randomly from 4 bags during bagging and then subjected to a methodology previously developed to separate agglomerated particles after pelleting. After the particles' separation, particle size was analyzed. The experiment consisted of 4 mash treatments (control) and a 2 x 4 factorial arrangement (2 pellet die x 4 particle sizes), totaling 12 treatments with 4 replicates per treatment. All the samples were analyzed using the ASABE method S319.4 with the addition of sieving agent to prevent particles agglomeration during the analysis. Data were statistically evaluated using the GLM procedure of JMP and means they were separated by Tukey's HSD with a statistical significance considered at $P < 0.05$. Pellet diameter did not influence the degree of grinding during pelleting ($P > 0.05$). As corn particle size increased, the particle size of the mash diets increased (936, 1251, 1387, and 1567 μm ; $P < 0.05$). The degree of grinding during pelleting increased as mash particle size increased regardless of the pellet die diameter (936 vs. 598 and 603 μm), (1251 vs. 755 and 742 μm), (1387 vs. 913 and 818 μm), (1567 vs. 877 and 871 μm) when the initial corn particle size was 855, 1659, 2043, and 2534 μm , respectively. The results of this study indicated that pellet diameter did not influence the degree of grinding during pelleting. However, as mash particle size increased, the degree of grinding during pelleting increased.

Key Words: pellet; particle size; roller mill; pellet die

95 *In-ovo* injections of glutamine and sodium butyrate regulate intestinal epithelium development in a broiler intestinal organoid model. Liang-en Yu^{1*}, Peter Mann¹, Lydia Schlitzkus¹, Federico Ghiselli², Yihang Li¹, ¹University of Delaware, Newark, Delaware, United States, ²University of Bologna, Bologna, Italy.

Early life events play significant roles in the tissue development and animal's health in their later life. Early nutrition, through *in ovo* delivery, has shown beneficial effects on improving intestinal health in broiler chickens. However, the underlying mechanism is not fully

investigated. The proliferation and differentiation of intestinal epithelial stem cells (IESCs) and progenitor cells govern the rapid development and functions of intestinal epithelium. A recently developed 3D intestinal organoids culture technique allows investigations on intestinal epithelial functions that are close to the physiological conditions. In this study, we evaluated the effects of *in ovo* feeding of two well-known gut beneficial nutrients, glutamine (Gln) or sodium butyrate (SB), on intestinal epithelial development and functions by using intestinal enteroids culture. A hundred eggs of commercial Cobb500 broilers were injected with 0.2 ml of either PBS, 3% Gln, or 1%SB on day E18. Chicks were euthanized at the day of hatch. The small intestines were sampled for epithelial isolation and enteroids culture. The enteroids formation and growth were monitored by microscope. After 4 days of culture, the enteroids were harvested for FITC-dextran (FD4) permeability analysis, and gene expression analysis of epithelial cell markers and functional proteins. One-way ANOVA and Student's T test were used for statistical analysis. Our results indicated that *in ovo* injection of both Gln and SB significantly ($P < 0.05$) increased the enteroids formation rate compared with PBS control. *In ovo* SB injection significantly ($P < 0.05$) increased the gene expressions of nutrient transporters (B0AT1, SGLT1, EAAT3) and tight junction proteins (TJP1, ZO2) compared with the PBS control group. Moreover, both Gln and SB treatments significantly ($P < 0.01$) reduced the FD4 permeability of the cultured enteroids. The gene expressions of epithelial cell markers were analyzed. In comparison with PBS control, ChgA (enteroendocrine cell) was upregulated ($P < 0.1$) in Gln group, MUC2 (goblet cells) was upregulated ($P < 0.05$) in SB group, while LYZ (Paneth cells) was down regulated in SB group, Lgr5 (IESCs) were numerically downregulated in both of Gln and SB groups. Together, our results suggested that the *in ovo* injection of Gln or SB simulated intestinal epithelium proliferation, and programmed the epithelial cell differentiation towards absorptive cells, which may improve the early post-hatch feed adaptation. Understanding the programming effects of early nutrition would shed lights on developing novel feeding strategies for improving gut health.

Key Words: Gut health; Butyrate; Enteroids; in-ovo feeding; Glutamine

96 Evaluating the effects of long-term storage on egg quality parameters, moisture loss, and fatty acid content of eggs from laying hens fed corn-soybean meal-based diets with different levels of nonphytate phosphorus. Grace Perrin^{1*}, Michael Ford¹, Anthony Pescatore¹, Michael Azain², Sunday Adedokun¹, ¹University of Kentucky, Lexington, Kentucky, United States, ²University of Georgia, Athens, Georgia, United States.

This study investigated the effects of long-term storage on egg quality, moisture loss, and fatty acid composition of white leghorn eggs. A corn-soybean meal-based diet with

three levels of nonphytate phosphorous (NPP; 90, 100, and 110% of requirement) was fed to 252 hens in a randomized complete block design with location as the blocking factor. Birds received stimulatory light at 16 wk of age. Average BW at 24 wk of age were 1.46, 1.45, and 1.47 kg/bird, respectively, for birds fed diets containing 90, 100, and 110% of the requirement for NNP. The three dietary treatments were fed from 12-24 weeks of age with nutrient and energy requirements adjusted for age. Two sets of eggs were collected at the end of week 8 of lay (week 24 of age). One set was analyzed at day 0 and the other set was weighed and stored at 4°C before analysis on day 45. Measured egg quality parameters included egg weight, yolk weight, albumen weight, shell weight, yolk percent, albumen percent, shell percent, egg breaking strength, yolk color, albumen height, and Haugh unit. Liquid contents of two eggs per replicate were freeze-dried, ground, and analyzed for fatty acid (FA) composition. Egg weight decreased ($P < 0.05$) with storage while yolk percentage and the intensity of yolk color increased ($P < 0.05$). Storage resulted in lower ($P < 0.05$) albumen height and Haugh unit. The 90% NPP treatment had higher ($P < 0.05$) yolk percentage compared to eggs from hens fed diet containing 100% NPP. Diet with 110% NPP resulted in higher ($P < 0.05$) polyunsaturated and total unsaturated FA. Storing eggs for 45 days at 4°C resulted in an increase in monounsaturated FA. This study demonstrates that storing eggs for 45 days at 4°C will lead to a decrease in egg weight, yolk percentage, and decrease albumen quality (Haugh unit and height). Storing eggs for 45 days also resulted in an increase in the intensity of yolk color. Increasing the dietary level of NPP by 10% resulted in an increase in the proportion of polyunsaturated and total FA.

Key Words: egg quality; egg storage; fatty acid; nonphytate phosphorous; moisture loss

97 A comparative study to determine the effect of breed and feed restriction on glucose metabolism of chickens. Pengfei Du^{1*}, Xiangli Zhang¹, Huanjie Wang¹, Xiuwen Shi¹, H.Y Zhang¹, W Chen¹, Y.Q. Huang¹, ¹*College of Animal Science and Technology, Henan Agricultural University, Zhengzhou, China.*

The glucose metabolism of birds draw widely attention due to nearly twice the fasting blood glucose of mammals. The

aim of this study is to explore the effects of breed and feed restriction on the glucose metabolism of chickens. In experiment (Expt.) 1, the mixed-sex famous fast-growing breed Arbor Acres (AA) broilers and Chinese traditional medicinal chicken with black skin and a slow growth rate (Silky chickens) were selected to study the effects of breed on chicken glucose metabolism (conventional feeding). Expt. 2 was then performed to evaluate the determine the effect of feed restriction on glucose metabolism of chickens. In this study, the mixed-sex AA broilers was allowed to feed to 7 d *ad libitum*, then those birds were randomly divided into restricted feeding group (RG) and a free feeding group (FF) group. The RG feeding time changed as 8:00 to 13:00 in 7-21 d. From 22 to 42 d, all birds were free access to food and water. At 21 and 42 d of age, glucose tolerance test (GTT, $n = 10$) was performed, i.e., after 16 h of fasting, blood glucose was measured at different time points after the chickens were given 2 g/kg of glucose solution orally, then these birds were sampling, pancreatic and liver tissues were collected for gene expression ($n = 8$). After assessing normal distribution and homogeneity of variances, data were analyzed by unpaired t-test, one-way analysis of variance (ANOVA). The results showed that AA broilers had a relatively slow rise of blood glucose to oral glucose solution than the Silky chickens on 21 and 42 d ($P < 0.05$), it probably attributes to lower expression of pancreatic insulin (*INS*), and higher transcription of phosphoenolpyruvate carboxy kinase 1 (*PCK1*) and glucose transporter 2 (*GLUT2*) in liver ($P < 0.05$). Response to feeding restriction from 7 to 21 d, the fasting blood glucose and the response speed to oral glucose in AA broilers were increased on 21 d ($P < 0.05$), and the serum glucose concentrations after 3 weeks compensatory growth on 42 d were improved by early feed restriction. Feeding restriction could also upregulate the mRNA level of pancreatic *INS* on 21 and 42 d, as well as decrease the expressions of *PCK1*, glucose-6-phosphatase catalytic(*G6PC*), and *GLUT2* in liver on 21 d ($P < 0.05$). These results revealed that Silky chickens have a stronger ability to regulate glucose homeostasis than AA broilers, and feed restriction could improve the AA broilers fasting blood glucose and the response to oral glucose.

Key Words: feed restriction; pancreas; Silky chickens; AA broilers; glucose tolerance

Student Competition: Immunology, Health and Disease III

98 Validation of a novel foam vaccination system for chicks in the hatchery. Kenneth Elliott^{1*}, Hong Li¹,
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Spray vaccination is a method of mass vaccination used to vaccinate day-old chicks against infectious bronchitis virus (IBV) and new castles disease virus. Despite advances over 40 years, spray technology can still suffer from a lack of uniform vaccine application to chicks under manufacturer recommended application. A novel foam vaccination system was designed and developed to deliver vaccines through edible foams. We hypothesized that foams can be used as a vaccine vehicle to improve the uniformity of vaccination while maintaining the level of protection. Using IBV as a disease model, a vaccination trial took place with foam vaccination being compared to eyedrop and spray vaccination on account of uniformity of application and level of protection provided. Day-old chicks received the same dose of IBV vaccine through eyedrop, spray, or foams. There were two foam treatments with one group of chicks vaccinated with one pass of full dose vaccine and the other group with two passes of half dose vaccine. Spray and eyedrop vaccination with a full dose of vaccine were carried out according to the manufacturer's instructions. The chicks were raised for 26 days. Nasopharyngeal swabs were performed on day 6 for RT-PCR analysis. Blood samples were collected on day 21 for ELISA tests. Tracheal ring samples and tracheal swabs were collected day 26 for histopathological and VI analysis. RT-PCR showed that eyedrop vaccination and 2-pass foam application had 100% coverage, 1-pass foam had 92.3% coverage, and spray had 72% coverage. The ELISA ranked from highest to lowest titer was 2-pass foam, eyedrop, 1-pass foam, and spray. Tracheal swabs and histological evaluation also support foam vaccination as a potential alternative to spray vaccination. The results of the study support the idea that foam vaccination can apply IBV vaccine uniformly to birds and maintain the protective level of spray vaccination. Using foam vaccination in the industry would ensure that flocks are well protected against infectious disease while they are being raised on the farm.

Key Words: chicken; hatchery; infectious bronchitis virus; vaccination; foam

99 Oxidative stress as a potential pathogenic cofactor for skeletal disorders in broilers. Yuguo Tompkins^{1*}, Guanchen Liu¹, Brett Marshall¹, Woo Kim¹,
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The genetic selection for heavy muscle gain and fast growth in broilers has been linked to increased prevalence of bone diseases and leg problems. Meanwhile, coccidiosis caused by *Eimeria* spp., the most economically impactful poultry disease, has been associated with not only the increased oxidative stress but also poor bone quality. Oxidative stress

occurs under conditions where excess free radicals are generated in sufficient amounts to cause tissue damage. Our prior studies have indicated that *Eimeria* infected birds had high levels of oxidative stress and lower antioxidant capacity in bone marrow. Besides, *Eimeria* infected birds had lower bone mineral content, density, and slower bone formation. However, the relationship between *Eimeria*-induced oxidative stress and bone health is unclear. In order to further understand the interaction between oxidative stress and bone metabolism, we utilized *in ovo* and *in vitro* models of induced oxidative stress. For *in vitro* studies, chicken primary mesenchymal stem cells (MSCs) were isolated from 1-day old chicks and exposed to hydrogen peroxide (H₂O₂) to induce oxidative stress. Cellular ROS level were measured by DCFDA / H2DCFDA assay, and cellular expression of key osteogenic genes were measured by qRT-PCR. Induction of oxidative stress on primary MSCs resulted in an inhibition in osteogenesis (*RUNX2* and *SPPI*; $p < 0.05$). For *in ovo* studies, injection of H₂O₂ into the dorsal aorta was used to induce systemic oxidative stress. The viability of embryos was confirmed by the heartbeat rate, and only alive embryos samples were collected. Key osteogenic and osteoclastogenic genes, reactive oxygen species and oxidative stress marker genes were measured by qRT-PCR. One-way ANOVA was performed, and means were compared by Tukey's test when ANOVA indicated significance. The *in ovo* H₂O₂-induced oxidative stress decreased the expression of osteogenic related marker genes (*BMP2*, *BGLAP* and *RUNX2*; $p < 0.05$) and increased expression of genes related to oxidative stress (*CAT*, *SOD* and *iNOS*; $p < 0.05$) without altering the lipid oxidation in embryos. Taken altogether, our studies demonstrate that the oxidative stress is one of the key factors that mediate lower bone quality by suppressing bone formation in broilers during stress. Because it is well known that oxidative stress is associated with various infectious diseases and poor welfare status in poultry, this study contributes to solving the animal welfare and production issues including skeletal disorders for the poultry industry.

Key Words: osteogenesis; Oxidative stress; bone health; bone formation; MSCs

100 Role of stressors on disease progression in broiler breeder pullets during *Hisotomonas meleagridis* challenge. Catherine Fudge^{1*}, Justin Lowery¹, Mark Bursleson², Zohreh Mehdiপুর¹, Chongxiao Chen¹, ¹North Carolina State University, Raleigh, North Carolina, United States, ²Wayne Farms LLC, Dobson, North Carolina, United States.

Histomonas meleagridis infects both chickens and turkeys. However, turkeys commonly present with severe clinical signs while chickens are less affected. Producers have reported increased broiler breeder mortality in early growth due to *H. meleagridis* infections. The objective of this study

was to understand the role of common stress factors during broiler breeder production on morbidity and mortality from *H. meleagridis* infection. 480 YP x Ross 708 chicks were randomly assigned to one of 8 treatments (10 birds x 6 replicates) and raised in battery cages. They were fed the same pullet basal diets with different feeding programs: *ad libitum* feeding (ABD); skip a day feeding (SDF, D17-D28); daily restricted feeding (DRF, 75% amount of feed compared to SDF). Treatments were NC: SDF, PC: SDF; T1: ABD; T2: DRF; T3: SDF + 110g/ton salinomycin (D1-D28); T4: SDF+ Safe-Guard Aquasol fenbendazole at 3mg/kg BW (D14-D16); T5: SDF + infectious bursal disease, reovirus, and salmonella vaccination (D1); T6: SDF + cocci vaccination (D1). On day 14, PC and T1-T6 were cloacally inoculated with 100,000 histomonads/bird. Body weight (BW) was collected on D0, 14, and 28. Mortality was recorded throughout the trial. At D28, all birds were scored for signs of histomoniasis in the ceca and liver, using a 0-4 scale with 4 being the most severe; blood was taken from 1 bird/cage for blood chemistry analysis using an iSTAT1 analyzer; intestinal permeability was measured using FITC-d. Results were analyzed using one-way ANOVA in SAS 9.4. Significant differences were determined when $P < 0.05$. Duncan's Multiple Range Test was used for mean separation. Three of the six mortalities observed in this trial were due to histomoniasis, 2 in T2 and 1 in T4. T2 and T4 also had increased liver scores compared to PC ($P = 0.0098$). Body weight was significantly impacted by the feeding program with the highest in T1 and lowest in T2 ($P < 0.0001$) at D14 and D28. No difference in BW was seen between PC and NC. The FITC-d analysis indicated that T2 had significantly higher intestinal permeability compared to the rest of the groups ($P = 0.0093$), however, *H. meleagridis* infection did not affect intestinal permeability while comparing PC to NC. Lastly, no significant differences were seen in infection rates ($P = 0.7633$) and blood chemistry ($P > 0.05$) between treatments. In summary, histomoniasis alone did not impact birds BW or change gut permeability. Higher permeability in T2 may be due to insufficient nutrition for gut development. Liver scores were increased when daily feeding was reduced to 75% of NC or dewormer (fenbendazole 3 mg/kg BW), indicating that these stressors may contribute to disease progression and mortality.

Key Words: Growth performance; Broiler breeder; Histomoniasis; Intestinal permeability; Disease progression

101 Effects of *Eimeria maxima* oocyst dose on growth performance and intestinal health parameters in coinfection necrotic enteritis disease model using *E. maxima* and *Clostridium perfringens* in broiler chickens. Doyun Goo^{1*}, Janghan Choi¹, Hanseo Ko¹, Hyun Lillehoj², Woo Kim¹, ¹University of Georgia, Athens, Georgia, United States, ²USDA-ARS, Beltsville, Maryland, United States.

The aim of the current study was to investigate the effects of the oocyst doses of *Eimeria maxima* on growth performance and intestinal health parameters in broiler chickens afflicted by necrotic enteritis (NE). Necrotic enteritis was induced using an established coinfection disease model using *E. maxima* and *Clostridium perfringens*. A total of 360 1-d-old male Cobb 500 broiler chickens were allotted to 6 groups with 6 replicates. Six different treatments were consisted with NC; a group with non-challenged, T0⁺; a group challenged with *C. perfringens* 1 x 10⁹, T5K⁺; T0⁺ + 5,000 *E. maxima* oocysts, T10K⁺; T0⁺ + 10,000 *E. maxima* oocysts, T20K⁺; T0⁺ + 20,000 *E. maxima* oocysts, and T40K⁺; T0⁺ + 40,000 *E. maxima* oocysts. All groups were orally infected using *E. maxima* strain 41A on d 14 and *C. perfringens* strain Del-1 on d 18 (4 days post infection: dpi). The experiment lasted for 28 days and samples were collected from the euthanized chickens for analyses of intestinal health on d 19, 20, 21, and 28. All data were analyzed using R software. Results showed that T10K⁺, T20K⁺, and T40K⁺ groups decreased in their body weights (BW) ($p < 0.001$) at d 21 (7 dpi), and reduced body weight gain (BWG) and feed intake (FI) at d 14 to 21 compared to NC and T0⁺. Except for NC, increasing dosage with *E. maxima* decreased d 21 BW, d 14 to 21 BWG and FI (linear; $p < 0.001$ and quadratic; $p < 0.001$), and increased d 14 to 21 feed conversion ratio (FCR) (linear; $p < 0.001$). However, there were no effects on BWG, FI, and FCR from d 21 to 28. Results of intestinal permeability using FITC-dextran showed that T40K⁺ treatment had increased intestinal permeability than NC and T0⁺ treatments on d 20 (6 dpi) ($p < 0.01$). Increasing oocyst numbers of *E. maxima* increased intestinal permeability on d 19, 20, and 21 (5, 6, and 7 dpi) (linear; $p < 0.05$), but there was no effect on d 28 (14 dpi). Jejunal lesion scores (scale from 0 to 4) showed that T20K⁺ and T40K⁺ treatments on d 19 and 20 (5 and 6 dpi) increased ($p < 0.05$) the jejunal lesion scores compared to NC, but had no effects on d 28 (14 dpi). On d 21 (7 dpi), increasing *E. maxima* doses increased water contents of ileal digesta and feces (linear; $p < 0.01$) and *C. perfringens* colony count of ileal digesta (linear; $p < 0.05$). Fecal *E. maxima* oocyst count increased on d 20 and 21 (6 and 7 dpi) (linear; $p < 0.001$ and quadratic; $p < 0.001$), but there was no statistical difference in d 19 (5 dpi). In conclusion, the dosages of *E. maxima* oocysts significantly enhanced the severity of NE, and negatively affected growth performance and intestinal health from 6 to 7 dpi. However, all the treatment groups recovered by d 28.

Key Words: *Clostridium perfringens*; *Eimeria maxima*; Necrotic Enteritis; Broiler chicken; Intestinal health

102 The impact of coccidiosis and necrotic enteritis on the microbiota of chickens – a meta-analysis. Andrea Pietruska^{1*}, Cristiano Bortoluzzi², Ruediger Hauck¹, ¹Auburn University, Auburn, Alabama, United States, ²DSM Nutritional Products, Kaiseraugst, Switzerland.

Coccidiosis in chickens is caused by the protozoan parasites *Eimeria* spp. The infection provides a growth advantage to the bacterium *Clostridium perfringens* (CP), frequently leading to necrotic enteritis. Both enteric diseases cause important economic losses in the poultry industry, due to bad performance and expenses for treatment and prevention. Changes in customer demand towards residue-free products, strict drug regulations, and drug resistances make it necessary to search for alternative treatments. One approach is to improve the bacterial composition in chickens to prevent and fight enteric diseases. The influence of coccidiosis and necrotic enteritis on the microbiota of the chickens was investigated in several studies in the recent years. The aim of this research was to summarize the data of these studies in a meta-analysis to provide a basis for future research. Inclusion criteria for studies on microbiota in chickens were that experiments contained a group infected with one or both pathogens and an uninfected control group, the use of 16SrRNA Illumina sequencing and the public availability of raw data. The data of each study was individually pre-processed in qiime2 for consistency. Meta-analyses of three different datasets were performed: one on raw data of nine experiments on chickens infected with coccidia only; the second on data of four studies on chickens infected with CP only; the third on raw data of seven experiments with chickens infected with coccidiosis and necrotic enteritis. The meta-analysis of relative abundance and alpha diversity of the datasets was performed in R using the SIAMCAT and metafor package. Potential confounding factors that were considered were days post infection, intestinal segment, further treatments, and *Eimeria* spp. In all three sets the relative abundance of *Proteobacteria* and *Firmicutes* was changed the most by infection with the pathogens. The infection with coccidia changed the relative abundance in 31 families, infection with both pathogens in 15 families, infection with CP in 2 families. Analysis of the variance revealed that the impact of coccidia on the microbiota is stronger than the co-infection with CP. CP alone has a larger effect on the variance compared to the confounding factors than coccidia infection alone. Alpha diversity was not affected by infection with either pathogen or combination of *Eimeria* sp. and CP. In conclusion, the heterogeneity of these microbiota studies makes recognizing common trends difficult, although it seems that coccidia infection affects the microbiota more than an infection with CP does. Future studies should focus on the bacterial functions that are changed due to these infections using metagenome techniques.

Key Words: *Clostridium perfringens*; microbiome; *Eimeria*; meta-analysis; poultry

103 Evaluating mRNA abundance of cytokines and chemokines in the modern broiler and heritage breed during a necrotic enteritis challenge. Laney Froebel^{1*},

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Necrotic enteritis (NE) is an enteric disease caused by *Clostridium perfringens* toxins and is estimated to cost the global poultry industry \$6 billion annually. Modern and heritage birds tend to exhibit differential responses to disease challenges. In this comparative study, we evaluated mRNA abundance of cytokines and chemokines in the peripheral blood and spleen of Athens Canadian Random Bred (ACRB) and Cobb broilers in response to subclinical NE. The design was a 2×2 factorial with breed (ACRB and Cobb) and challenge (no challenge and NE) as main factors. On day of hatch, 96 male chicks (48 ACRB and 48 Cobb) were allocated to the four experimental treatments with 8 replicate cages and 3 birds/cage. On d 14, birds in the NE-challenged groups were orally gavaged with ~3,000 *Eimeria maxima* sporulated oocysts followed by two doses of approximately 1×10⁸ CFU of *C. perfringens* on d 19 and d 20. On d 21, blood and spleen samples were collected to measure mRNA abundance of interleukin (IL)-1β, IL-10, IL-18, tumor necrosis factor (TNF)-α, C-C chemokine ligand (CCL)-5 and CCL20. Additionally, birds were necropsied and scored for intestinal NE lesions. Data were analyzed by 2-way ANOVA (JMP Pro 16) and significance ($P \leq 0.05$) between treatments was evaluated by LSD test. The NE-challenged Cobb birds exhibited greater ($P \leq 0.05$) mRNA abundance of IL-1β, IL-10, IL-18, TNF-α, CCL5, and CCL20 in peripheral blood compared to all other treatment groups. While mRNA abundance of IL-10 and CCL5 was not significantly different ($P > 0.05$) in the spleen between treatment groups, abundance of IL-1β, IL-18, TNF-α, and CCL20 was greater ($P \leq 0.05$) in the ACRB compared to the Cobb birds. Lesion scores were not significantly different ($P > 0.05$) between ACRB and Cobb birds. Collectively, NE-challenged Cobb broilers exhibited a greater proinflammatory (IL-1β, IL-18, TNF-α, CCL5, and CCL20) and regulatory (IL-10) responses in peripheral blood, indicating a more acute response to NE compared to ACRB birds. Further, greater mRNA abundance of IL-1β, IL-18, TNF-α, and CCL20 in the spleen of ACRB birds could indicate different lymphatic system responses to NE compared to Cobb birds. Further detailed research of the immune responses of heritage and modern breeds is warranted to better characterize their responsiveness to this challenging enteric disease.

Key Words: ACRB; *Clostridium perfringens*; chemokine; cytokine; Necrotic enteritis

104 Effects of *Clostridium perfringens* and *Eimeria maxima* co-infection on intestinal morphology and stem cell differentiation in broiler chickens. Sydney Kinstler^{1*}, John Maurer¹, Eric Wong¹, Charles Hofacre², Matthew Jones³, Margie Lee¹, ¹*Virginia Polytechnic Institute and State University, Christiansburg, Virginia, United States*, ²*Southern Poultry Research Group, Inc., Watkinsville*,

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Necrotic enteritis is a significant intestinal disease in the broiler chicken industry that affects producer economics and animal welfare annually. NE is caused by *Clostridium perfringens* but is predisposed by coccidiosis when infected with the parasite *Eimeria*, resulting in reduced feed intake, weight loss, and mortality. Our central hypothesis is *C. perfringens* and *Eimeria* co-infection damages the intestinal epithelium, preventing nutrient absorption by rupturing enterocytes and birds respond to intestinal atrophy through upregulation of stem cell proliferation and differentiation to rebuild villi and restore function. A NE challenge model used 144 male chicks placed in 12 Petersime battery cages and assigned to 1 of 4 treatments; 1. Non-challenged control, 2. *Eimeria* infection, 3. *Clostridium perfringens* infection, 4. *Eimeria* and *C. perfringens* co-infection. Chickens were inoculated with 1,000 sporulated oocysts of *Eimeria maxima* on d14 and 1×10^8 cfu *C. perfringens* on d19. At peak infection, d21, 6 chickens/treatment were weighed, necropsied, and gross lesions scored on a scale of 0 to 3. In-situ hybridization was performed on jejunum sections from 4 chickens/treatment using stem cell marker *Olfm4* to measure morphology characteristics. Gene expression in jejunum mucosa was analyzed by qPCR using 6 chickens/treatment and data were log transformed. Genes included *Olfm4*, inflammation markers *NOX1*, *NFKB1*, and *MyD88*, absorptive cell markers *Notch1*, *Atoh1*, and *PepT1*, and secretory cell markers *LRP5* and *Muc2*. Tukey HSD was used to analyze normally distributed data and Steel-Dwass All Pairs test was used for non-parametric analysis. An increase in crypt depth (CD), decrease in villus height (VH), and decrease in VH:CD was observed in co-infected chickens ($P < 0.01$). Crypt depth increased in chickens infected with *Eimeria* or co-infection compared to the non-challenged control and *C. perfringens* infection ($P < 0.01$). Villus height was blunted in chickens that received an infection, particularly co-infection ($P < 0.01$). Stem cell proliferation and subsequent absorptive cell differentiation was not affected by *Eimeria* infection or co-infection. *C. perfringens* infection upregulated secretory cell differentiation and inflammation ($P < 0.01$). Body weight decreased in co-infected chickens ($P = 0.01$) and lesion scores of 1 were exclusively observed in this group, indicating a subclinical infection. Inoculation of NE pathogens to induce infection or co-infection did not affect stem cell proliferation or differentiation. However, differences in morphology decreased body weight after co-infection, suggesting that nutrient absorption is affected after intestinal epithelium damage, impacting growth performance.

Key Words: *Clostridium perfringens*; necrotic enteritis; *Eimeria*; stem cells; morphology

105 Vaccines using *Clostridium perfringens* sporulation proteins prevent chicken necrotic enteritis.

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Clostridium perfringens is a prevalent enteric pathogen responsible for chicken necrotic enteritis (NE). The mechanism of *C. perfringens*-induced NE remains unclear, while the bacterial toxins are implicated in NE. The objective of this study was to investigate whether vaccines using *C. perfringens* sporulation proteins would prevent NE. To assess the objective, sporulation proteins were prepared and evaluated for signaling pathways (inflammation and cell death) *in vitro* and NE challenge *in vivo*. The sporulation supernatant proteins (CP-spor-super1&2) from two *C. perfringens* isolates were marked as vaccines CP1 and CP2. Meanwhile, *C. perfringens* soluble proteins (CP-super1&2) prepared from the vegetative growth bacterial medium were used as control for mechanism exploration. Toxin types of these two *C. perfringens* isolates were detected by PCR. Induction of inflammatory response in epithelial CMT-93 and macrophage Raw 264.7 cells were performed. The presence of CPE and cell death signal pathways were evaluated by Western Blot. For chicken vaccine experiment, birds were immunized at d 0 and 10 with different doses of CP1 and CP2, and then infected with 20,000 sporulated *Eimeria maxima* M6 oocysts at d 16 and 10^9 CFU/bird *C. perfringens* at d 20. Chicken body weight was measured at d 0, 16, 20 and 21. Birds were euthanized at d 21 to collect blood, ileal tissue and content for analysis of antibody titer, histopathology, inflammation and *C. perfringens* colonization levels. mRNA levels of proinflammatory genes, mouse *Il1 β* , *Tnfa*, *Cxcl2* and chicken *Ifn γ* , *Il1 β* , *Il8-1*, were determined using real-time PCR. Differences between treatments were analyzed using One-way ANOVA followed by Bonferroni pairwise comparison using Prism 7.0 software. Data were also analyzed using the nonparametric Mann-Whitney U test. Experiments were considered statistically significant if *P* values were < 0.05 . *C. perfringens* enterotoxin (CPE) was detected positive for gene by PCR and protein by Western Blot in the CP-spor-super1&2. CP-spor-super1 or 2 induced cell death in CMT-93 and Raw 264.7 cells. CP-spor-super1 or 2 also induced inflammatory gene expression and necrosis in the Raw cells. Birds immunized with CP-spor-super1 or 2 were resistant to *C. perfringens* induced severe clinical NE on histopathology and body weight gain loss during d 20-21 (NE phase). CP-spor-super1 vaccine increased the chicken antibody titer in blood, but reduced NE-induced proinflammatory *Ifn γ* gene expression as well as *C. perfringens* luminal colonization and tissue invasion in the small intestine. Together, this study showed that CP-spor-super vaccines reduced NE histopathology and productivity loss and can be used as vaccine candidates for NE prevention.

Key Words: *Clostridium perfringens*; growth performance; Necrotic enteritis; inflammation; sporulation

Student Competition: Metabolism and Nutrition, Feed Additives II

106 The effects of a blend of botanical extract on production performance and egg quality of laying hens housed in a furnished cage system. Taiwo Makinde^{1*}, Stephanie Cotte², Bertrand Medina², Janice MacIsaac¹, Deborah Adewole¹, ¹Dalhousie University, Truro, Nova Scotia, Canada, ²Probiotech International Inc., St-Hyacinthe, Quebec, Canada.

Certain plant extracts are considered as natural health products because of their growth-promoting, antioxidant, anti-inflammatory, and antimicrobial properties in poultry. However, no study has examined the effects of a blend of plant extracts on both egg quality and performance characteristics in laying hens housed in furnished cages. This study investigated the effects of Phytozen® Liquid, a commercially formulated blend of botanical extracts (BEs), on the performance and egg quality of laying hens in a furnished cage system. A total of 280 Lohmann Lite laying hens (38-week-old) were randomly allocated to either BEs (40 ml Phytozen® Liquid in 200 L of water) or control treatment group (without BEs in water) for 16 weeks (4 x 4-week periods, each period consisting of 3 wk of measurement + 1 wk washout period). Each group had 4 furnished cages with 35 hens/cage. Performance characteristics were recorded daily (feed weight, hen-day egg production-HDEP, egg weight) and weekly (feed intake, water intake, and feed conversion ratio), while egg quality parameters (eggshell breaking strength, albumen height, albumen weight, yolk weight, and eggshell weight) were measured periodically. All data were analyzed as repeated measures in 3 weeks crossover period design using the Statistical Analysis Software (S.A.S., version 9.4). The In-water supplementation of BEs significantly ($P < 0.05$) reduced water consumption in Period 2 (6.95%) and Period 4 (5.62%), feed intake in Period 1 (5.20%), and significantly ($P < 0.05$) improved the hen-day egg production in Period 4 (7.10%) compared to the control groups. However, BE-supplementation did not affect the feed conversion ratio and the average daily egg weight. On the other hand, the effect of BE supplementation significantly ($P < 0.05$) improved the internal egg quality parameters, including the albumen height in Period 2 (4.55%) and Period 3 (6.61%), and Haugh unit in Period 3 (3.03%). Meanwhile, the BE supplementation did not affect the egg breaking strength, albumen weight, percentage yolk weight, and eggshell weight. Moreover, the interaction between the treatment and the period was significant ($P < 0.001$) for the mean daily water intake, and marginally significant for feed intake ($P = 0.059$). These results suggest that the administration of BEs to laying hens up to 52 weeks of age improved some performance and egg quality metrics in furnished cages.

Key Words: laying hens; production performance; Egg quality; furnished cages; Phytozen

107 Growth performance, nutrient utilization, gut permeability and intestinal lesion scores in broilers fed diets with different dietary levels of fiber, protein, enzymes, prebiotics and challenged with or without *Eimeria* spp. Yang Lin^{1*}, Oluyinka Olukosi¹, ¹University of Georgia, Athens, Georgia.

A total of 784 Cobb 500 male broiler chicks were used in two 21-d experiments to study the possibility of exogenous enzymes or prebiotics to help recover growth performance and nutrient utilization in broilers challenged with *Eimeria* spp. In Expt. 1, total of 392 birds were allocated to 8 treatments in a 4×2 factorial arrangement (4 diets with or without *Eimeria* challenge). Each treatment had 7 replicates with 7 birds per replicate. The 4 diets were based on low fiber-low protein (LFLP) basal diets. The basal diet was supplemented with no enzyme (NE), protease alone (P), xylanase combined with protease (XP), or prebiotic xylo-oligosaccharides (XOS). Expt. 2 was similar in design but diets had high fiber and adequate protein (HFAP) and were supplemented with NE, xylanase alone (X), XP or XOS. Birds and feed were weighed on d 0, 15 and 21. The challenge group were inoculated with a solution containing oocysts of *E. maxima* (12500 oocysts), *E. tenella* (12500 oocysts), and *E. acervulina* (62500 oocysts) on day 15. Gut permeability was measured on 5-d post-infection (DPI) and the intestinal lesion was scored on 6 DPI. The comparison of treatments was subjected to two-way ANOVA. In Expt. 1, there was no significant enzyme × challenge interaction on growth performance. Birds receiving diets with P had greater ($P < 0.05$) weight gain (WG) and feed intake (FI) in pre-infection period. *Eimeria* challenge significantly ($P < 0.01$) decreased WG, FI, and gain: feed, whereas supplements had no effects in post-infection period. In Expt. 2, no significant interaction or main effects were found in growth performance response. *Eimeria* challenge decreased ($P < 0.01$) WG, FI and gain: feed. In both experiments, *Eimeria* challenge lowered ($P < 0.01$) DM and N digestibility and total tract retention of DM, N and AME. In Exp.1, P and XOS supplementation showed positive ($P < 0.05$) effects on AME and total retention of DM and N whereas supplements had no effect in Exp. 2. In addition, *Eimeria* challenge resulted in higher ($P < 0.01$) gastrointestinal leakage and severer lesion whereas supplements had no effect. In conclusion, protease or xylo-oligosaccharides supplementation helped recover AME and total retention of N when supplemented to birds receiving LFLP diet under *Eimeria* challenge.

Key Words: broiler; *Eimeria*; protease; xylanase; xylo-oligosaccharides

108 Evaluation of AviPower®2-500 on intestinal health parameters and broiler performance with a cocci vaccine program. Daniel De Leon^{1*}, Rosana Hirai¹, Austin

Silva¹, Garrett Powell¹, Macey Randig-Biar¹, Philip Maynard², John Smalley², Ester Grilli², Benedetta Tugnoli³, Audrey McElroy¹, ¹Texas A&M University, Bryan, Texas, United States, ²Vetagro, Inc., Chicago, Illinois, United States, ³Vetagro S.p.A, Reggio Emilia, Italy.

This experiment was conducted to determine the impact of varying inclusion of a microencapsulated botanical feed additive, AviPower®2-500 (AP), on performance, intestinal morphology, intestinal lesion scoring, litter moisture (LM), litter scoring (LS), fecal oocyst shedding, and footpad dermatitis (FPD). Ross 308 male broilers were fed an all-vegetable diet formulated to meet or exceed breed nutrient requirements from D0-49. Fourteen replicate pens of 29 chicks each were spray-vaccinated (Advent®) for coccidia and randomly allocated to 1 of 5 treatments (TRTS). Inclusion rates of AP included 0 ppm; 250 ppm (Half); or 500 ppm (Full). Feeding phases were Starter D0-14; Grower D15-28; Finisher D29-39; and a zero inclusion Withdrawal D40-49. TRTS included Negative control (NC); Half dosage of AP from starter-finisher (TRT 2); 0, Full, Half (TRT 3); 0, Full, Full (TRT 4); or Half, Full, Full (TRT 5). On D14, D28, D39, and D49, BW, BW gain (BWG), feed intake, and mortality corrected FCR were measured with feeding phase changes. On D14 and D28, intestinal samples were collected (1 bird/pen) for morphology. On D7, D21, and D28 3 birds/pen were evaluated for macroscopic intestinal lesions from *Eimeria* spp. and microscopic oocyst counts for *E. maxima*. On D14, D28, and D49, 5 samples of litter/pen were collected to measure LM, litter was scored, and 5 birds/pen were evaluated for FPD lesions. On D6-8, D13-15, and D20-22 fecal samples were pooled over a three-day period to evaluate oocyst shedding counts. Data was analyzed via SAS using the GLM procedure with a randomized complete block design and significance at $P < 0.05$. Tukey's range test was used for mean separation. From D14-28, TRT 4 resulted in improved FCR compared to NC with all other TRTS producing similar results, and all AP TRTS significantly improved BW and BWG compared to NC. Similarly, from D0-28, AP TRTS had improved FCR (TRT 5 was similar to NC), FI, BW, and BWG compared to NC. D39 BW was improved for all AP TRTS compared to the NC. D39 FI increased for TRT 5 when compared to the NC and TRT 4 with all other TRTS producing similar results. D0-39 BWG and FI was significantly improved by all AP TRTS when compared to NC. Overall, TRT 5 FI increased when compared to the NC. D28 LS was higher for TRTS 2, 4, & 5 when compared to the NC but had no differences at D49. D28 FPD was higher for TRT 5 when compared to the NC but had no differences at D49. No differences were seen for intestinal morphology or lesion scores, or fecal oocyst shedding. Results indicated the potential for AP to improve performance of broilers following coccidia vaccination, however future research could determine inclusion levels for different feeding phases for optimal programs.

Key Words: Intestinal Health; Coccidiosis; Growth Performance; Antibiotic Alternative; Botanicals

109 Effects of chitosan oligosaccharides on broilers challenged with a natural subclinical necrotic enteritis model. Emanuele Goes^{1*}, Weijuan Huang^{1,2}, Mi Zhou¹, Lingyun Chen¹, Leluo Guan¹, Doug Korver¹, ¹University of Alberta, Edmonton, Alberta, Canada, ²South China Agricultural University, Guangzhou, China.

The removal of dietary antibiotic growth promoters (AGP) increases the risk of necrotic enteritis (NE) in broilers, and effective replacements must be found. Dietary chitosan oligosaccharides (COS) of varying molecular weights (Mw) have been tested as potential AGP replacements in broilers. Previously we reported that medium Mw COS (95 KDa) increased broiler BW after an NE challenge and tended to increase BW during the entire cycle compared to birds fed a diet without AGP, although gross intestinal NE lesion scores were not affected. From that study, samples obtained from the most promising treatments were further evaluated to determine the COS effects on cecal microbial composition and short-chain fatty acids (SCFA), mortality and footpad dermatitis (FPD). Treatments were: COS 95 KDa included at 0.2 or 5 g/kg of the diet, a positive control (PC; with antibiotic and coccidiostat) and a negative control diet (NC; without medications). Each treatment was fed to 8 replicate pens of 18 birds each. A natural sub-clinical NE challenge model (oral 15X coccidiosis vaccine dose at 12 d, and 24-hour feed removal at 18 d) was applied to all birds. Cecal contents collected at 22 d were used to determine the bacterial profile and SCFA production. Mortality was recorded daily, and FPD was measured at 38 d. The microbial profile was analyzed by Orthogonal contrasts, and the remaining data were analyzed by ANOVA with means compared by Tukey's test at 5% probability. The mortality throughout the trial was 3.91%; 55.5% of that occurred between 18 to 24 d, similar to field cases of NE. Overall, the cecal microbial profile was not affected by treatment (by ANOVA). However, in pairwise comparisons, NC had a greater ($P < 0.01$) abundance of *Clostridium sensu stricto* 1 at the genus level than the COS treatments. At the species level, COS treatments had a higher abundance of *Lactobacillus oris* than NC ($P < 0.01$) and PC ($P < 0.01$), and a higher abundance of *Lactobacillus ingluviei* than PC ($P < 0.01$). PC resulted in more (4.79 $\mu\text{mol/ml}$; $P = 0.03$) propionate than 0.2 g/kg COS 95 KDa (2.63 $\mu\text{mol/ml}$) but was similar to 5 g/kg COS 95 KDa and NC (3.32 and 2.82 $\mu\text{mol/ml}$, respectively). Isobutyrate from the 5 g/kg COS 95 KDa group (0.58 $\mu\text{mol/ml}$; $P = 0.01$) was higher than in the PC (0.34 $\mu\text{mol/ml}$) and NC (0.37 $\mu\text{mol/ml}$) but was similar to the 0.2 g/kg COS 95 KDa. Dietary treatment did not affect FPD. COS 95 KDa may act as a prebiotic modulating the growth of *Lactobacilli* in broilers and enhance performance following an NE challenge. COS 95 KDa may be part of a strategy to replace AGP; however, further mechanistic studies are required.

Key Words: broiler chicken; chitosan oligosaccharide; : Antibiotic replacement; necrotic enteritis challenge model; microbial profile

110 Effects of dietary feed additives on the performance of broilers raised on used litter seeded with coccidiosis oocysts. Cooper Fritzlen^{1*}, Kim Wilson², Mike Persia¹, ¹*Virginia Tech, Blacksburg, Virginia, United States*, ²*Orffa, BV, Athens, Georgia, United States*.

An experiment was conducted to evaluate the effects of feed additives on the performance and responses to coccidiosis of broilers raised on used litter. Used litter was generated by housing 25 chicks that received a 10x CocciVac® B52 via the feed for the first 2 days and raised until 18 days of age to allow for oocysts to be shed into the litter. All litter was moved to the center of the building, mixed and evenly redistributed within the 3 days of downtime before the start of the experiment. Day old male Ross 708 broiler chicks (0.08 m² per bird) were housed in floor pens and randomly assigned to one of the four treatments: positive control (PC) fed a diet without additive and raised on clean pine shavings, negative control (NC) fed same diet and raised on used litter (NC), diet with 60 ppm salinomycin (SAL) and raised on used litter, and diet with 500 ppm Excential Alliin Plus (garlic and cinnamon extracts) and 250 ppm Excential Beta-Key a betaine product (APBK; Orffa Additives, B.V. Breda, Netherlands) and raised on used litter. In total, 12 replicate pens of 24 birds for each treatment. Body weight gain (BWG) and mortality corrected feed conversion ratio (FCRm) were reported for the 0-16 d, 0-29 d, and 0-42 d periods. On 11-13 d, 17-19 d, and 22-24 d, fresh excreta were collected from each pen to determine oocyst shedding. Data were analyzed using ANOVA with means separation using Fisher's LSD (JMP Pro 16; $P \leq 0.05$). Oocyst counts were lowest in PC (130, 1,400, and 15,580) and highest in NC (12,220, 107,380, 199,290), while SAL (6,190, 39,250, 119,400) and APBK (11,330, 65,020, 47,350) were intermediate for all three periods, respectively ($P \leq 0.05$). The NC resulted in reduced BWG of 36 g ($P < 0.01$), 85 g ($P = 0.01$) and 122 g ($P = 0.16$) in comparison to the PC birds at 16, 29 and 42 d, respectively. NC birds also resulted in a worsened FCRm of 1.354 vs. 1.320 ($P = 0.04$), 1.478 vs. 1.465 ($P = 0.58$) and 1.604 vs. 1.568 ($P = 0.10$) relative to PC birds from 0-16, 0-29 and 0-42 d, respectively. Mortality was low for both controls with 3.5% and 2.8% for the PC and NC, respectively. Treatment of broilers with APBK resulted in increased BWG of 33 g ($P = 0.01$), 97 g ($P < 0.01$) and 172 g ($P = 0.05$) and reduced FCRm 1.308 vs. 1.354 ($P < 0.01$), 1.426 vs. 1.478 ($P = 0.03$) and 1.551 vs. 1.604 ($P = 0.02$) relative to NC from 0-16, 0-29 and 0-42 d, respectively. The improvement in both BWG and FCRm with APBK were similar to both PC and Sal ($P > 0.05$). The reduced overall performance and increased oocyst shedding in the NC validated the mild coccidiosis model. The feed additives, APBK, were able to ameliorate negative performance effects with the reused litter model and may have reduced oocyst shedding.

Key Words: broiler; coccidiosis; feed additive; performance; oocyst shedding

111 The immunometabolic effects of sodium butyrate supplementation in the ileum of broiler chickens. Famatta Perry^{1*}, Cristiano Bortoluzzi³, Cinthia Eyng², Michael Kogut², Ryan Arsenault¹, ¹*University of Delaware, Newark, Delaware, United States*, ²*USDA-ARS, College Station, Texas, United States*, ³*Texas A&M University, College Station, Texas, United States*.

Butyrate is a four carbon short chain fatty acid and a product of microbial fermentation in the gut. Butyrate has been shown to have positive effects on broiler health via stimulation of anti-inflammatory responses in the gut. However, it has been proposed that butyrate is metabolized by intestinal cells via beta oxidation, reducing oxygen levels and regulating T-cells which can lead to inflammatory responses. To examine the immune and metabolic impact of butyrate in the gut, Cobb 500 by-product birds were fed the standard corn-soy meal with the treatment group receiving 0.1% sodium butyrate (SB) supplementation. Kinome peptide array analysis was performed on the ileum tissue of d21 birds. Cytokine gene expression of TNF alpha, interleukin (IL)-1 β , IL-6 and IL-10 were also performed on d21 ileum samples. P-values reported were less than 0.05 using paired t-test for the kinome peptide array and ANOVA for the cytokine gene expression. All statistical tests were performed by comparing SB supplemented group to control and are reported as such. The kinome peptide array results showed significant changes in the phosphorylation status of proteins involved in the major metabolic pathways, that is, glycolysis, tricarboxylic acid cycle and beta oxidation. Further analysis showed increased activity of beta oxidation enzymes including hydroxyacyl-coenzyme A dehydrogenase and acetyl-CoA carboxylase. The kinome results also showed differential changes in the phosphorylation status of proteins important in T cell receptor signaling, C-type lectin receptor signaling and HIF-1 signaling. Detailed analysis showed the major proteins in these pathways such as HIF-1 alpha, CD45, ZAP70, NFKB, PKC and mTOR showed increased activity compared to control. The gene expression data showed increased expression of IL-1 β and IL-10 in SB supplemented groups. The results of the kinome peptide array showed the immunometabolic effects of butyrate in the ileum of birds at d21. Butyrate increases beta oxidation which may induce hypoxia in the gut especially in segments with larger microbial populations like the ileum, indicated by increased HIF-1 and PKC activity. This increased hypoxia can trigger inflammatory responses via immune and immunometabolic pathways such as T-cell and C-type lectin signaling pathways. These pathways can stimulate the production of inflammatory cytokines, such as IL-1 β as shown in the cytokine expression results. However, the increased expression of IL-10 suggests that butyrate may also modulate excessive inflammatory responses via the stimulation of anti-inflammatory cytokines. Thus, the

immunometabolic regulation of butyrate in the gut involves complex processes.

Key Words: Butyrate; Gut Health; Immunometabolism; Kinome peptide array; Hypoxia

112 The effects of marker selection, mix time, batch size, and In-line near infrared spectroscopy (NIR) on the coefficient of variation (Mix Uniformity) of broiler diets. Andrea Rubio^{1*}, Corey Wishon¹, Clark Christopher², Shannon Conrad², Jeffrey Pope³, Adam Fahrenholz¹, ¹*North Carolina State University, Raleigh, North Carolina, United States*, ²*Bruker Corporation, Billerica, Massachusetts, United States*, ³*House of Raeford, Magnolia, North Carolina, United States*.

Previous research has reported that mixing uniformity is essential to maximize growth performance of animals with a low daily intake, such as poultry. Typically, an adequate mix time or uniformity has been considered to have been achieved when a defined nutrient or additive in the diet has a coefficient of variation (CV) $\leq 10\%$. However, differences in diet formulations, batch size, marker selection, and sample preparation could be sources of variation which may lead to over- or underestimation of mix uniformity. The objective of this study was to evaluate the effects of marker selection, mix time, batch size, and In-line near infrared (NIR) spectroscopy on mixer CV of broiler diets. A total of 12 batches of feed were mixed with 3 replicate batches/treatment. Feed was produced using the NC State University Feed Mill Education Unit's 2 Ton counterpoise ribbon mixer. The batches of feed were mixed for 4.5 min (3 min dry mix and 90 s of wet mix) and 30 s (0 s dry mix and 30 s wet mix) to obtain a uniform and a non-uniform mix, respectively. The experiment constituted a $2 \times 2 \times 4$ factorial arrangement of 2 mix times, (4.5 and 0.5 min), 2 batch sizes (1 and 2 Tons), and 4 methodologies to evaluate mixer performance (sodium chloride, Microtracers (Red#40 and Blue#40), and In-line NIR). Ten samples were collected at equally spaced time intervals from the mixer discharge conveyor and analyzed for CV using multiple tracers (chloride ion concentration using Quantab Test Strips, Microtracer F-Red#40(count), and Microtracer F-Blue#40(count)). Spectra readings were obtained with an In-line NIR at the mixer discharge conveyor to generate the proximate analyses used for CV calculations. Data were statistically evaluated as the aforementioned $2 \times 2 \times 4$ randomized factorial arrangement. For main effects, there were no statistical differences between batch sizes on CV ($P > 0.05$). However, a mix time of 4.5 min and the In-line NIR generated CVs less than 10% compared to 0.5 min, sodium chloride, and the Microtracers. Interactions were apparent ($P < 0.05$) only for methodology and total mix time. The sodium chloride method reported CV values greater than 10% regardless of mix time. The results of this experiment indicated that mixer CV differed depending on total mix time and methodology used.

Key Words: broiler; uniformity; coefficient of variation; In-line NIR; mix

113 The effects of feeding whole-in-shell peanuts, sweet potatoes, and/or high-oleic peanuts to laying hens on ileal nutrient digestibility. Kari Harding^{1*}, Dimitri Malheiros¹, Thien Vu², Rebecca Wysocky¹, Ramon Malheiros¹, Kenneth Anderson¹, Ondulla Toomer², ¹*North Carolina State University, Raleigh, North Carolina, United States*, ²*ARS, USDA, Raleigh, North Carolina, United States*.

As the demand for corn and corn by-products continues to rise, so does the cost of chicken feed resulting in increased costs of chicken products for the consumer. Therefore, the search for locally grown alternative ingredients has become a priority to recapture nutrients for animal feed, removing ingredients from the waste stream and minimizing the cost of grains and importing them. Previous research has shown that sweetpotatoes and high-oleic peanuts could be acceptable alternatives, but little is known about their nutrient digestibility. Our research aim was to determine the effects of feeding whole-in-shell peanuts, sweetpotatoes, and/or high-oleic peanuts to laying hens on ileal digestibility. Five isocaloric, isonitrogenous treatments were used in this study, a conventional control (C1), an 8% high-oleic peanut (HOPN), a 4% sweetpotato (SWP), a 4% whole-in-shell peanut (WPS), and a 4% SWP + 4% HOPN diets. Ninety-six laying hens were housed in single bird cages and randomly assigned a treatment with sixteen replicates per treatment and fed their respective diets for six weeks. 2% Celite was added to each treatment diet as a tracer to determine digestibility. Fecal samples were collected one week before termination and ileal samples were collected from each bird on the day of termination. All data were analyzed using a one-way analysis of variance with $P < 0.05$ as significance. The apparent metabolizable energy corrected for nitrogen (AMEn) was significantly greater ($P < 0.05$) in HOPN as compared to the other treatments. C1 and SWP had equivalent AMEn levels and were higher than both WPS and SWP + HOPN. The apparent nitrogen retention percentage (ANR) was higher ($P < 0.05$) in SWP + HOPN diet compared to WPS and SWP, however was not significantly different than HOPN and C1 ($P < 0.05$). The apparent fat digestibility (AFD) was greater ($P < 0.05$) in SWP + HOPN than all other treatments with SWP being slightly lower. The HOPN had the lowest ($P < 0.05$) AFD percentage of all treatments, with WPS being greater. SWP diet had significantly greater ($P < 0.05$) apparent protein digestibility (APD) than all other treatments with HOPN being the next highest. C1 and SWP + HOPN were not significantly different but were significantly higher ($P < 0.05$) than WPS. From these results, feeding HOPN and SWP separately could be beneficial because they have similar, or exceed the levels of AMEn, and APD compared to C1. However, if feeding WPS, a supplementation of another energy source and protein source may be beneficial to achieve similar digestibility results to C1.

Key Words: Digestibility; High-Oleic Peanuts; Alternative Feed Ingredients ; Sweet Potato; Whole-In-Shell Peanuts

114 Effects of a direct fed microbial fed from 0 to 70 weeks of age on laying hen performance and egg quality from 18-70 weeks of age. Jordyn Samper^{2*}, Miloud Araba¹, Miguel Ruano¹, Troy Lohrmann¹, Mike Persia², ¹Quality Technology International, Inc, Elgin, Illinois, United States, ²Virginia Tech, Blacksburg, Virginia, United States.

Direct fed microbials (DFM) have been used to improve animal health and performance in response to reduced antibiotic use within the poultry industry. An experiment was conducted to determine the effects of a DFM on laying hen production and egg quality. Production parameters included Hen-Day egg production (HDEP), egg weight (EW), egg mass (EM), feed intake (FI), and feed conversion ratio (FCR). Egg quality parameters included internal content weights, Haugh unit, shell weight, specific gravity, and shell breaking strength. These results are for the laying phase of a comprehensive study on feeding a DFM to laying hens from hatch until 70 weeks of age. The DFM, Q-Biotic® 1DP, is a *Bacillus subtilis* strain. Two treatments were evaluated including a control without DFM (Con) and the same control diet with 300,000 CFU of DFM per g of feed (DFM). Hens were fed a controlled amount of feed throughout the experiment, based on breeder recommendations and egg production. No differences were recorded in FI, with hens consuming 98.1 g per day ($P = 0.56$). Dietary treatments were fed to 12 replicate groups of six Hy-Line W-36 caged laying hens resulting in 144 hens. Production parameters were measured on a 4-week basis beginning at 24 weeks when hens initiated consistent EP. Egg quality was measured every 8 weeks from 26 to 50 weeks, every 4 weeks from 50 to 66 weeks, and every 2 weeks from 66 to 70 weeks. Data were analyzed with ANOVA using repeated measures in JMP Pro 16 (SAS Institute Inc., Cary, NC). If ANOVA was significant ($P \leq 0.05$), LS-means were separated using a Student's t-test. Initial BW was not different at 17 weeks ($P = 0.59$) with both weighing 1.23 kg, regardless of treatment. Supplementation of the DFM increased HDEP resulting in 86.0% and 84.1% production for DFM and Con hens, respectively ($P = 0.02$). Con hens did produce heavier EW (60.7 g vs 60.1g) throughout the production cycle ($P \leq 0.01$). The increased HDEP and reduced EW however, resulted in no differences in EM ($P = 0.12$) although DFM treatment was numerically higher. There was a 7.1 pt improvement ($P = 0.06$) in FCR, with a 2.016 for the DFM-fed hens and 2.087 in the control hens. No differences were noted in hen BW over the laying phase ($P = 0.14$). Egg shell quality responses were mixed as DFM hen eggs showed a trend in improved specific gravity ($P = 0.07$), but no differences were recorded in egg shell breaking strength ($P = 0.14$) or shell weight ($P = 0.91$). Overall, supplementation of the DFM to laying hens increased HDEP and improved FCR when fed from hatch to 70 weeks of age.

Key Words: DFM; FCR; laying hen; Bacillus Subtilis; Q-Biotic 1DP

115 Effect of *Bacillus subtilis* and its delivery route on hatch and growth performance, blood biochemistry, and immune status of broiler chickens. Samson Oladokun^{1*}, Deborah Adewole¹, ¹Dalhousie University, Truro, Nova Scotia, Canada.

As the poultry industry recedes from the use of antibiotic growth promoters (AGP), there is a need to evaluate possible alternatives and the delivery method that maximizes their effectiveness. This study evaluated the effect of a probiotics (*Bacillus subtilis* fermentation extract) and its delivery route (in-feed or *in ovo*) on hatch and growth performance, blood biochemistry, and immune status of broiler chickens. A total of 1,860 Cobb 500 hatching eggs were incubated for 21 days. On d 12 of incubation, viable eggs were randomly allotted to 4 groups: the non-injected, *in ovo* saline (S), *in ovo* *B. subtilis* 1 (P1), and *in ovo* *B. subtilis* 2 (P2). On d 18 of incubation, S, P1, and P2 groups received 0.2 mL saline diluent, 10×10^6 , and 20×10^6 CFU of the bacterium/0.2 mL saline diluent via the amnion, respectively. At hatch, chicks were re-allotted to 5 new treatment groups: P1, P2, 0.005% in-feed *B. subtilis* extract (F), 55 mg/kg in-feed bacitracin methylene disalicylate (BMD), and corn-wheat-soybean diet negative control (NC) in 9 replicate pens (22 birds/pen) and raised for 35 d. Hatch parameters were assessed on d 0 and body weight and feed intake (FI) were measured weekly. On d 25, 1 bird/cage was euthanized, and immune organs weighed. Blood samples were collected for plasma biochemistry and serum immunoglobulins (IgG, IgM) analysis. Data were analyzed in a randomized complete block design using the generalized linear model. Treatments recorded no effect on chick weight, chick length, and hatchability. Contrastingly, birds in treatments F and P2 had reduced ($P < 0.050$) FI at week 5, compared to the NC treatment. However, no change in average body weight gain (ABG) and feed conversion ratio (FCR) were observed during the same period. At the end of the trial (d 35), while BMD treatment showed a tendency ($P = 0.087$) to increase average FI compared to the NC treatment, ABG and FCR were similar for all treatment groups. Plasma electrolytes minerals (sodium and chloride) levels were increased ($P < 0.05$) by the BMD treatment compared to the NC treatment. Other treatment groups recorded intermediate values of these electrolyte minerals. While the relative weights of the bursa and spleen were not affected by treatments, all probiotics treatments (irrespective of route and dose) reduced ($P < 0.001$) the levels of serum IgG compared to the NC treatment. However, the greatest reduction (99%) was observed in the P2 treatment. This study suggests that probiotics (especially *in ovo* delivered) might offer better immunological protection to broiler chickens by neutralizing pathogenic organisms that could lead to the production of natural antibodies, without negatively affecting hatch and growth performance.

Key Words: broiler chicken; in ovo; performance; probiotics; immune status

116 Performance of Japanese quail (*Coturnix coturnix japonica*) supplemented with different levels of organic acids. Ashvini Bansod^{1*}, ¹IVRI, Bareilly, Uttarpradesh, India.

This research study was undertaken to know the performance of Japanese quail (*Coturnix coturnix japonica*) supplemented with different levels of organic acids. The day old chicks were randomly and equally distributed into 5 treatments: T₀ (basal diet as per ICAR 2013), T₁ (T₀ + 0.2% Acetic acid), T₂ (T₀ + 0.2% Butyric acid), T₃ (T₀ + 0.2% Propionic acid), and T₄ (T₀ + 0.2% Acetic acid + 0.2% Butyric acid + 0.2% Propionic acid) with 60 birds in each group having 4 replicates of 15 birds each. It was observed that the experimental birds of T₄ group fed with diet containing blends of organic acids had shown maximum weight as compared to other treatment groups. The treatment group T₄ exhibited numerically better weekly feed efficiency as compared to other treatment groups. In terms of albumin, globulin and total protein, cholesterol and triglyceride statistical differences ($p < 0.05$) were observed. Dressing percentage was numerically highest in T₂ group (67.81%). The value of ileal pH was lowest in T₄ group (5.52). The highest value of intestinal length was recorded in treatment group T₀ and lowest value observed in T₂. Highest intestinal weight was in T₂ group whereas lowest intestinal weight was in T₄. The value of *E. coli* and *Salmonella* was lowest in T₄ group. In terms of digestibility, DM digestibility was statistically highest ($p < 0.05$) in T₁ group and nitrogen retention was highest in T₃ group. Higher profit per kg of quail was observed in treatment group T₁ as Rs. 41.17/- and lowest in control group i.e. T₀ as Rs. 40.41/-. The data was analyzed by utilizing Statistical Package for the Social Sciences (SPSS) Version 17.0. The differences between means were subjected to ANOVA by univariate analysis using General Linear Model. On the basis of results obtained in the present study it could be concluded that the performance in Japanese quail fed with blends of organic acids exhibited a positive impact on growth, feed efficiency, blood biochemical, carcass characteristics, gut parameter and metabolic traits. Further the supplementation of blends of organic acids was found to be beneficial for profitable quail production.

Key Words: Performance; Quail; Carcass; Biochemical; Digestibility

117 Effect of dietary plant essential oils on production performance, egg quality and intestinal barrier function of post-peak laying hens. Hao Yuan^{1*}, Yongshuai Wang¹, Leilei Wang¹, H.Y Zhang¹, Y.Q. Huang¹, W Chen¹, ¹Institute of Animal Science and Technology, Henan Agricultural University, Zhengzhou, China.

Emerging evidence indicated that the natural plants and their extracts as feeding additives play important roles in

maintaining livestock health including plant essential oils, implying that dietary plant essential oils supplementation might improve the production performance, egg quality, and intestinal barrier function in post-peak laying hens. To confirm the hypothesis, a total of 384 350-d-old Hyline female birds were assigned to 3 diet treatment for 6 weeks: Corn-wheat commercial diet (Ctrl), Ctrl + 100 mg/kg plant essential oil (EOA, including 13.5% Carvacolol, 4.5% Cinnamaldehyde, and 2% Holly oil), or Ctrl+100 mg/kg plant essential oil (EOB, including 13.5% thymol and 4.5% Cinnamaldehyde). Feed intake, egg production, and egg mass were determined daily, and the feed conversion rate (FCR) was calculated. Four eggs were collected from each replicate at the end of week 2, 4 and 6 of the experiment for egg quality determination. Each replicate was randomly selected one bird and sampled, the duodenum and ileal mucosa was collected for intestinal barrier gene expression determination. The data was analyzed by One-way the analysis of variance (ANOVA) followed by Tukey multiple comparisons. The results showed that the FCR in EOB decreased at 1 to 3 weeks when compared to Ctrl group ($P = 0.08$). The diet with EOA and EOB significantly increased the egg yolk index at weeks ($P < 0.01$). The expression levels of the genes associated with the intestinal barrier were determined by RT-PCR and showed that the gene expressions of Cadherin-1 in groups EOA and EOB were significantly increased in the duodenum ($P = 0.01$), and the gene expression of Zona Occludens 1 ($P < 0.05$) and Claudin-1 ($P < 0.01$) in the ileum were remarkably increased when compared to Ctrl group. Collectively, these results indicated that supplementation of 100 mg/kg plant essential oil could decrease the FCR, egg yolk index, and intestinal barrier function in post-peak laying hens.

Key Words: egg quality; laying hen; performance; intestinal barrier; plant essential oil

118 Replacing corn in diets with wheat shows effects on performance, nutrient digestibility, and hepatic health of broilers. Bin Wei^{1*}, Leilei Wang¹, H.Y Zhang¹, Yilu Wang¹, Y.Q. Huang¹, W Chen¹, ¹Institute of Animal Science and Technology, Henan Agricultural University, Zhengzhou, Henan, China.

It is established that both wheat and corn contain large amounts of starch (77% vs. 72%, respectively), but they differ with the proportion of starch digested in domestic birds. Increasing available energy content of the diets of broiler has the potential for improved efficiency of nutrient utilization, which would improve performance through increased metabolizable nutrient supply. Therefore, this study was to evaluate the effects of replacing corn with wheat on digestibility of nutrients, growth performance, and liver health in broilers. A total of 480 healthy day-old male AA broilers were randomly divided into one of three treatment groups with eight replications, having twenty animals each, for 39 d experimental period excluding the

adaption period from 1 to 10 d. Birds were fed a starter (11-21d) and finisher (22-39 d) diet as pellet. The levels of replacement were 15%, 30%, and 55% in starter diets, and 25%, 40%, and 63% in finisher diet in control (Ctrl), mild, and high replacement group, respectively. Parameters were evaluated regarding growth performance, digestibility, and hepatic health. The data was analyzed by One-way the analysis of variance (ANOVA) followed by Tukey multiple comparisons. Dietary high replacement of corn with wheat has resulted in an increased feed intake and a decrease in weight gain significantly ($P < 0.05$) as compared to Ctrl and mild replication group, and thus increased the ratio of feed intake to gain during 11 to 39 d ($P < 0.05$). Reflecting to nutrient digestibility, with the replication corn in diets with wheat, the crude protein, dry matter, crude fat, and the crude ash were significantly decreased ($P < 0.01$). Meanwhile, along with a higher area or smaller diameter of steatosis vacuoles, the diet with mild and high replacing corn with wheat increased aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activity in plasma ($P < 0.01$), respectively. Besides compared to Ctrl, the expression of tumor necrosis factor alpha (*TNF- α*) was notably upregulated, and the serum immunoglobulin G was significantly decreased in those birds fed high wheat diets (both $P < 0.05$). Overall, the higher replacing corn in diets with wheat (55% and 63% in starter and finisher diets, respectively) compromised the growth performance though decreasing nutrient digestibility, which companied with hepatic health of broilers.

Key Words: broiler; corn; growth performance; wheat; hepatic health

119 The impact of dietary fermented calcium butyrate and *Clostridium butyricum* on the performance, egg quality and intestinal barrier function in post-peak laying hens. Yongshuai Wang^{1*}, Leilei Wang¹, Chuanchen Su¹, Ziyang Wang¹, Huaiyong Zhang¹, Y.Q. Huang¹, W Chen¹, ¹*College of Animal Science and Technology, Henan Agricultural University, Zhengzhou, China.*

With the continuous egg production, the production performance and egg quality of laying hens is gradually decreased in post-peak laying hens. In this context, this experiment was conducted to investigate the effect of dietary fermented calcium butyrate and *Clostridium butyricum* (1.0×10^9 cfu/kg) on production performance, egg quality, and the intestinal barrier of in post-peak laying hens. 384 of 50-weeks-old Hyline variety brown female birds were randomly assigned to 3 diet treatment with 8 replicates (16 birds each diet) for 6 weeks: corn-soybean commercial diet (**Ctrl**), Ctrl+300 mg/kg fermented calcium butyrate, Ctrl+300 mg/kg *Clostridium butyricum*. Egg production, egg weight and feed intake were recorded every day. 4 eggs were collected from each replicate at the end of week 2, 4 and 6 of the experiment for egg quality determination. One bird from each replicate was randomly selected and sampling, the ileum mucosa was collected for intestinal barrier gene expression determination. The data was analyzed by One-way the analysis of variance (ANOVA) followed by Tukey multiple comparisons. Results showed that dietary fermented calcium butyrate increased the average egg weight when compared to Ctrl group ($P < 0.05$). The supplementation of *Clostridium butyricum* increased the albumen height and Haugh unit ($P < 0.05$) at the end of 2 week, the yolk index at the end of 4 week, and the yolk ratio at the end of 6 week when compared to Ctrl birds (all $P < 0.05$). The average egg weight ($P < 0.01$) of birds fed the diets with calcium butyrate or *Clostridium butyricum* were significantly increased at the end of 4 week than those received Ctrl diets. In addition, the expression levels of *Occludin* ($P < 0.01$) and *Mucin2* ($P < 0.05$) of ileum mucosa were significantly increased by dietary calcium butyrate or *Clostridium butyricum* administration relative to Ctrl group. In conclusion, these results indicated that dietary supplementation of 300 mg/kg fermented calcium butyrate or 300 mg/kg *Clostridium butyricum* could improve the production performance, egg quality, or intestinal barrier function in post-peak laying hens.

Key Words: laying hens; performance; *Clostridium butyricum*; intestinal barrier; fermented calcium butyrate

Student Competition: Metabolism and Nutrition, Enzymes

120 Broiler performance and nutrient digestibility from 1 to 42 days of age was influenced by nutrient density and supplementation of a multicarbohydrase containing α -galactosidase. Joseph Gulizia^{1*}, Jose Vargas¹, Susan Bonilla¹, Jose Hernandez¹, Leopoldo Almeida¹, Patricia Carvalho¹, Sureerat Thuekeaw², Sara Llamas Moya³, Tri Duong³, Wilmer Pacheco¹, ¹*Auburn University, Auburn, Alabama, United States*, ²*Chulalongkorn University, Pathumwan District, Bangkok, Thailand*, ³*Kerry, Global Technology and Innovation Centre, Naas, Ireland*.

This study evaluated the effect of nutrient density and a multicarbohydrase containing α -galactosidase enzyme (CAG) on broiler performance and nutrient digestibility from 1 to 42 d of age. A total of 1,800 d-old Ross 708 x YPM male broilers were distributed randomly into 72 floor pens. Each pen contained 25 birds and was assigned to 1 of 6 treatments, with 12 replicates per treatment. Three basal diets were formulated to contain two energy levels (standard (SE) and -100 kcal/kg ME (LE)) and two AA densities (90 and 96%) based on Ross 708 nutrient specifications. The first basal diet contained SE with 96% AA density (SE96AA), the second diet contained LE and 96% AA density (LE96AA), and the third diet contained LE and 90% AA density (LE90AA). Three additional treatments were manufactured by supplementing 0.2 g/kg of CAG to each basal diet. Feed intake and BW were determined at 14, 28, and 42 d of age, and FCR was corrected for mortality. Ileal digesta was collected from 5 birds per pen on d 42 to assess nutrient digestibility. Data were analyzed as a 3 x 2 (nutrient density x CAG inclusion) factorial arrangement using the GLM procedure of SAS. Means were separated using Tukey's HSD with statistical significance considered at $P \leq 0.05$. Broilers consuming SE96AA had 5.6% higher BW and 9-points lower FCR ($P < 0.05$) than broilers fed LE90AA. Broilers that consumed CAG supplemented diets had lower FCR from 1 to 28 d ($P < 0.05$) compared to diets without CAG, but no differences were observed from 1 to 42 d. There was an interaction between nutrient density and CAG on BW and FCR at 42 d ($P < 0.05$). Broilers fed SE96AA supplemented with CAG had lower BW ($P < 0.05$) and 2.7-point numerically higher FCR. However, in LE96AA and LE90AA, the supplementation of CAG numerically lowered FCR 2.5 and 3.2-points, respectively. Broilers supplemented with CAG had a 4.7, 18.2, and 6.2% higher CP, calcium, and phosphorus digestibility, respectively, compared to broilers fed diets without CAG ($P < 0.001$). Broilers fed a CAG supplemented diet had a 106 kcal/kg higher apparent ileal digestible energy (AIDE) compared to broilers fed a non-CAG diet ($P < 0.001$). The highest improvement in AIDE (237 kcal/kg) was observed when CAG was supplemented to LE90AA compared to non-supplemented LE90AA ($P < 0.05$). Overall, supplementation of CAG improved nutrient digestibility of

broilers, but BW and FCR were influenced mainly by nutrient density.

Key Words: broilers; carbohydrase; energy; nutrient density; α -galactosidase

121 Evaluation of the effects of adaptation diet and exogenous carbohydrase enzymes on true metabolizable energy, cecal microbial ecology, cecal short-chain fatty acid profile, and cecal enzyme activity in roosters fed barley or rye diets. Annalise Anderson^{1*}, Mike Bedford², Carl Parsons¹, ¹*University of Illinois at Urbana-Champaign, Urbana, Illinois, United States*, ²*AB Vista, Marlborough, United Kingdom*.

Three experiments were conducted to evaluate the effect of adaptation diet and exogenous β -glucanase and xylanase on the TME_n of barley and rye. Single Comb White Leghorn roosters were fed adaptation diets based on corn/soybean meal, barley/soybean meal with and without β -glucanase, or rye/corn/soybean meal with and without xylanase for 4 wk. In Experiments 1 and 2, after the adaptation period, TME_n was determined using the precision-fed rooster assay for 100% barley or 100% rye diets with or without β -glucanase or xylanase, respectively. The TME_n assay consisted of fasting for 26 h, tube-feeding 30 g of the test diet (5 roosters per diet), and another 48 h of fasting during which excreta were quantitatively collected. Experiment 3 consisted only of feeding the adaptation diets for 4 wk (6 roosters per diet). Cecal samples were collected at the end of each study for analysis of microbial ecology, short-chain fatty acid (SCFA) profiles, and β -glucanase and xylanase activity. Data were analyzed using the one-way ANOVA for completely randomized designs as either 3x2 or 3x3 factorials, with adaptation diet and TME_n treatment as main effects. Significance was assessed at $P < 0.05$. The results of Experiments 1 and 2 indicated that β -glucanase significantly ($P < 0.05$) increased TME_n of barley and xylanase numerically increased TME_n of rye, but there was no significant effect of adaptation diet on TME_n values. For cecal microbial profiles, total *Eubacteria* and *Ruminococcaceae* were decreased ($P < 0.05$) and *Escherichia coli* was increased ($P < 0.05$) at the end of the TME_n assay compared with values obtained at the end of the adaptation period (with no TME_n assay). For SCFA, there was a large decrease ($P < 0.05$) for most SCFA at the end of the TME_n assay compared with the end of the adaptation period. For cecal enzyme activity, both β -glucanase and xylanase activity were increased for adaptation diets containing the respective enzyme. In Experiment 3, there were no consistent effects of adaptation diet on cecal microbial profiles or SCFA but cecal β -glucanase activity was increased ($P < 0.05$) by exogenous β -glucanase for barley and cecal xylanase activity was increased ($P < 0.05$) by exogenous xylanase for rye. Overall, the results of this study indicated that the precision-fed

rooster assay was sensitive for detecting effects of β -glucanase on TME_n of barley. Adaptation diet did not significantly influence the TME_n response to the enzymes, possibly because cecal fermentation (based on SCFA) was greatly reduced by fasting during the TME_n assay. Cecal β -glucanase and xylanase activity, however, were often increased by feeding high barley and high rye diets with inclusion of exogenous enzymes.

Key Words: barley; carbohydrase; xylanase; rye; β -glucanase

122 Evaluation of the efficacy of a mono-component single protease enzyme (DSM046) on growth performance, energy and nitrogen ileal digestibility, and gut health. Froylan Abraham Soriano Rodriguez^{*1,3}, Carlos López¹, Ernesto Ávila¹, José Arce², and Silvestre Charraga³, ¹Universidad Nacional Autónoma de México, ²Universidad Michoacana de San Nicolás de Hidalgo, ³DSM Nutritional Products México.

The use of proteases has proved beneficial effects on crude protein digestibility, body weight gain and feed conversion in commercial poultry production. The objective was to evaluate the efficacy of a sfericase protease (DSM046) with a phytase and xylanase on performance, gut health, litter quality, ileal digestibility, and skin pigmentation in broilers. A total of 1,800 one-day-old (Cobb 500) male chickens were allocated in 36 experimental pens in a completely randomized design with 4 treatments and 9 replicates until 42 days of age. Treatments were: 1) positive control (100% requirements); 2) negative control (-0.736% CP, -0.04% dig Lys, -0.039% dig Met+Cys, -0.048% dig Thr and -0.034% dig Arg); 3) as T2 with 10,000 NFP (New Feed Protease Units)/kg of feed; 4) as T2 with of 30,000 NFP/kg of feed. All the diets included 1000 FYT (Phytase Units)/kg of feed and 200 FXU (Fungal Xylanase Units)/kg of feed, considering its recommended nutritional matrix. The diets were based on corn, soybean meal, canola meal, DDGS, meat and bone meal and corn gluten meal. The analysis variables were body weight (BW), feed intake, feed conversion rate (FCR) and mortality, at 22 days of age ileal digestibility for energy and nitrogen, intestinal morphology (villi length and width, number) and absorption area capacity were measured. Footpad dermatitis was measured at 34 and 41 days of age (DSM Standard Protocol and Welfare Quality® 2009). At 41 days of age skin pigmentation, total and ammoniacal nitrogen in litter and environmental ammonia were measured. Data were subjected to ANOVA according to the GLM procedure, when statistically significant differences were observed ($P < 0.05$) Tukey test was performed ($\alpha = 0.05$). Differences were observed at 42 days of age ($P < 0.05$) for BW, T2 had lower value vs T1, T3 and T4. The higher FCR was obtained for T2 and was different than T3 and T1 ($P < 0.05$). Gut morphology showed differences for villi length and number, crypt depth and surface area (microns) ($P < 0.05$), T2 showed the lowest absorption capacity. T1 showed the lower energy

digestibility and the higher digestibility values were obtained for treatments with the addition of the protease (T3 and T4) ($P < 0.05$), no differences were reached for nitrogen digestibility between treatments ($P = 0.20$), results show the same tendency as energy digestibility. The treatments T1, T3 and T4 showed same skin pigmentation values and higher than T2 ($P < 0.05$). Results show that the addition of a recent developed mono-component single sfericase protease added in combination with a phytase and a xylanase has an additional positive effect in BW, FCR, intestinal villi morphology, ileal digestibility, and skin pigmentation in male broilers.

Key Words: digestibility; gut health; protease; skin pigmentation; enzyme combination

123 Efficacy of multi-component enzymes on growth performance of young chicks. Gerardo Abascal-Ponciano^{1*}, Jessica Starkey¹, Charles Starkey¹, ¹Auburn University, Auburn, Alabama, United States.

Previous studies with corn-soybean meal-based diets demonstrate that multi-enzymatic complexes (ENZ) increase the degradation of non-starch polysaccharides, compensate for reductions in energy and amino acids, reduce feed cost, and maintain optimal broiler performance. A randomized complete block experimental design was performed to evaluate the effects of 2 single fermentation multi-component enzymes (ENZA and ENZB) targeting xylans and cellulose at 4 concentrations on the performance of male Ross 708 x Yield Plus broilers from d 6 to 22. The 14 experimental corn-soybean meal-based treatments were: 1) a positive control diet (PC) with phytase at 500 FTU/kg of feed (PHY), formulated to meet bird nutritional requirements, 2) a mid-control diet (MC) with a 50 Kcal/kg reduction in energy + PHY, 3) a negative control diet (NC1) with a 100 Kcal/kg reduction in energy + PHY, 4) NC1 + ENZB at 1,000 XU/kg (NC-A), 5) NC1 + ENZB at 3,000 XU (NC-B), 6) NC1 + PHY (NC2), 7) NC2 + ENZA at 1,000 XU/kg (ENZA1), 8) NC2 + ENZA at 2,000 XU/kg (ENZA2), 9) NC2 + ENZA at 3,000 XU/kg (ENZA3), 10) NC2 + ENZA at 4,000 XU/kg (ENZA4), 11) NC2 + ENZB at 1,000 XU/kg (ENZB1), 12) NC2 + ENZB at 2,000 XU/kg (ENZB2), 13) NC2 + ENZB at 3,000 XU/kg (ENZB3), and 14) NC2 + ENZB at 4,000 XU/kg (ENZB4). On d of hatch, chicks ($n = 1,440$) were randomly allotted to 280 raised floor pens with 5 birds/pen and 20 replicate pens/treatment. Means were separated with the PDIF option of SAS V9.4 PROC GLIMMIX at $P \leq 0.05$. On d 0, chick BW was similar among all treatments ($P = 0.7634$). At d 22, broilers from PC and MC treatments had greater BW than all treatments except for those fed the ENZA2 diet ($P < 0.0001$). The lowest FI was reported in birds fed ENZB1 treatment but was similar to NC1, ENZA4, and ENZB3 fed broilers ($P = 0.0457$). Broilers fed PC and MC had similar BWG (833 and 824 g, respectively), ENZA1, ENZA2, ENZA3, and ENZB2 were able to gain similar weight as MC treatment, but PC differed from all

other treatments ($P < 0.0001$). Broilers fed the PC diet had the lowest FCR (1.3496; $P < 0.0001$), followed by MC-fed broilers (1.3883) but these were similar to ENZA2, ENZA3, ENZA4, and ENZB3. Broilers fed ENZA3 diet had the lowest numerical FCR of all ENZ diets, surpassing the NC1 treatment by 6 FCR points (1.4034 vs. 1.4580) and NC2 treatment by 2 points (1.4034 vs. 1.4247). In conclusion, birds fed ENZA2 and ENZA3 diets achieved similar BWG and FCR as the MC treatment.

Key Words: phytase; enzymes; xylanase; broiler growth performance

124 The effect of Allzyme® Spectrum supplementation on low nutrient wheat-soybean meal diets. Megan Bauer^{1*}, Sunday Adedokun¹, Tuoying Ao¹, Marquisha Paul¹, Jacqueline Jacob¹, Mike Ford¹, Anthony Pescatore¹, ¹*University of Kentucky, Lexington, Kentucky, United States.*

Feed accounts for up to 70% of the total cost of raising poultry. The addition of exogenous enzymes to poultry feed allows for greater extraction of nutrients from feed for digestion. Allzyme® Spectrum (Alltech, Inc., Nicholasville, KY, USA) is an enzyme complex including carbohydrase and phytase. Growth performance of broiler chickens fed wheat-soybean meal-based diets with reduced metabolizable energy, calcium, and available phosphorus (negative control; NC) plus 4 inclusion levels of Allzyme® Spectrum (0, 150, 200, 250 g/Ton) during the pre-starter (d 0-9) and starter (d 9-22) phase was evaluated in this study. A positive control (PC) treatment group having a commercial equivalent level of energy (3,050 kcal/kg), Ca (0.8%) and avP (0.4%) was included in the study. The NC diet consisted of a reduction of 90 kcal/kg ME and 15% Ca and P. A total of 300 d-old male broiler chicks were assigned to the 5 treatments in a randomized complete block design with 10 replicate cages of 6 chicks. Data were analyzed using the GLM procedures of SAS (v 9.4). Orthogonal contrasts were used to compare the PC and the NC and PC vs 150, 200, 250 g/Ton Allzyme® Spectrum. Orthogonal polynomial contrasts were utilized to determine polynomial effects of dietary supplemental levels of Allzyme® Spectrum on the performance of chickens. The chickens fed NC diet had lower ($P < 0.01$) weight gain (38.9 vs 48.7 g/bird/d), feed intake (47.8 vs 53.3 g/bird/d) and gain to feed ratio (0.606 vs 0.673 g/g) compared to those fed PC diet in entire 22d growing period, while the chickens fed diet supplemented with 150, 200, 250 g/Ton Allzyme® Spectrum had the equivalent weight gain, feed intake, and weight gain to feed ratio compared to those fed PC diet. Feed intake of chickens from d 9-22 (67.1-76.2 g/bird/d) and 0 to 22 (47.8-53.1 g/bird/d) linearly ($P < 0.05$) increased with increasing supplemental level of Allzyme® Spectrum. There was a quadratic effect ($P < 0.05$) of enzyme supplementation level on d 9-22 weight gain and d 0-9 gain to feed ratio peaking at 47.2 g/bird/d and 0.832 g/g, respectively, with 150g/ton Allzyme® Spectrum. Based on

overall weight gain (peaked at 35.1 g/bird/d) and gain to feed ratio (peaked at 0.66 g/g) of chickens in the 22d trial period, the optimal supplemental level of Allzyme® Spectrum in the diet was 150 g/Ton and 200 g/Ton, respectively. In conclusion, dietary Allzyme® Spectrum supplementation optimized growth performance of broiler chickens fed a wheat-soybean meal-based diet with reduced nutrients.

Key Words: broiler; enzyme; wheat; reduced nutrients; performance

125 Effects of corn kernel hardness, drying temperature and stimbiotic supplementation on broiler performance and gastrointestinal development. Joaquin Joaquin Cabanas-Ojeda^{1*}, Jhon Mejia-Abaunza¹, Paula Lozano-Cruz¹, V.L Aragao-Neto¹, Muhammad Ali¹, Maria Alfaro-Wisaquillo¹, Gustavo Quintana-Ospina¹, Lina Peñuela-Sierra^{1,3}, Gilson Gomes², Edgar Oviedo-Rondón¹, ¹*North Carolina State University, Raleigh, North Carolina, United States*, ²*AB Vista, Plantation, Florida, United States*, ³*Universidad del Tolima, Ibagué, Tolima, Colombia.*

Endosperm kernel hardness and post-harvest drying temperatures have been reported to affect corn nutrient composition and quality, and non-starch polysaccharide content. Several studies found beneficial effects of carbohydrase supplementation on broiler performance. Stimbiotic (STB) products that combine xylanase with fermentable oligosaccharides have shown positive effects on BW gain, feed intake, and FCR. Consequently, this study evaluated the effects of two yellow dent corn varieties with average and hard kernel hardness dried at 35 and 120°C, and supplementation of a STB (Signis®, AB Vista, Marlborough, UK) on broiler live performance and gastrointestinal development. The average and hard endosperm corn varieties were planted under similar conditions. At harvest, each variety was split into two groups to be dried at 35°C and 120°C. Eight treatments resulted from a 2 x 2 x 2 factorial arrangement with two corn kernel hardness, two drying temperatures and the inclusion of sand or STB (100 g/ton). A total of 1,920 Ross 708 male day-old chicks were placed into 96 floor pens and fed a starter (900 g/chicken), grower (2,000 g/chicken) and finisher (*ad libitum*) pellet diets up to 42d. Gastrointestinal organs were collected on empty BW at 42 d. Data were analyzed in a randomized complete block design and means separated using Tukey's and student's t-test. No three-way interaction effects were observed ($P > 0.05$) for all parameters evaluated. Two-way interaction between corn variety and STB was observed ($P < 0.05$) on BW and feed intake. Lower feed intake was observed between 1-14 and 1-42 d for chickens fed the hard corn variety. Consequently, BW was lower at 14 and 28d for chickens fed diets containing this cultivar. However, when STB was added feed intake increased and BW was similar to the other treatments. Drying temperatures and STB interacted on FCR ($P < 0.05$) and adjusted FCR ($P < 0.001$) at 14d only. The

STB improved FCR only for chickens fed diets with corn dried at 120°C. Endosperm hardness also affected FCR and FCR adjusted by mortality ($P < 0.05$) at 28 and 42 d. FCR was lower for the hard corn variety or any corn dried at 120°C ($P < 0.01$). Longer small intestines and ceca were observed ($P < 0.05$) in broilers fed any corn dried at 120°C at 42d. When corn was dried at 35°C, broilers had heavier ceca in the average hardness corn ($P < 0.05$). Larger livers and less visceral fat were obtained in broilers fed corn dried at 35°C ($P < 0.05$). In conclusion, corn variety and drying temperatures caused variation in broiler performance, adding stimbiotic significantly improved feed intake, BW in hard endosperm corn, and FCR at 14 d by 22 points in corn dried at 120°C. Some of these effects could be related to gut development.

Key Words: corn; Broilers; xylanase; kernel hardness; xylo-oligosaccharides

126 Investigating the effects of a stimbiotic supplementation along with wheat bran in corn and wheat-based diets on performance, ileal digestible energy, caecal short chain fatty acids and gut morphology in broilers. Shravani Veluri^{1*}, Mike Bedford², Gemma Gonzalez Ortiz², Oluyinka Olukosi¹, ¹University of Georgia, Athens, Georgia, United States, ²AB Vista, Marlborough, Wiltshire, United Kingdom.

A 42-day experiment was conducted to investigate the interactive effects of a stimbiotic (SB) and wheat bran (WB) in broiler chickens receiving corn or wheat-based diet. A total of 960-day old Cobb 500 broilers were allocated to 64 pens with 8 treatments and 8 replicates with 15 birds per replicate. The treatments were arranged in a 2×2×2 factorial, the factors being: diet (corn-SBM or wheat-SBM); SB (- or +); and WB (0 or 5%). Bodyweight gain (BWG), feed intake (FI), and FCR data were taken for starter (d 0 to 10), grower (d 10 to 28), and finisher (d 28 to 42) phases. Sample collection was done on d 18 and 42. Data was analyzed in a 2×2×2 factorial using mixed model of JMP. There was no significant 3-way interaction for any of the responses measured throughout the experiment except for ileal digestible energy (IDE) measured on d18. There was a significant SB×WB for FCR ($P < 0.05$) in the grower phase, SB supplementation improved ($P < 0.05$) FCR in the diets without WB. Both in the finisher and overall phases, birds receiving corn-based diets had higher ($P < 0.05$) FI and BWG compared with those receiving wheat-based diets. Gizzard pH tended to decrease with SB ($P = 0.06$) supplementation and was lower in wheat compared to corn-based diets on d18 ($P < 0.05$). Birds receiving diets with WB had higher jejunum pH on d 18 ($P < 0.05$). Ceca pH on d 42 was higher ($P < 0.05$) for birds on corn- compared with wheat-based diets. Total SCFA on d 18 and isobutyrate on d 42 was higher ($P < 0.05$) for corn- compared with wheat-based diets; however, on d 42, acetate, butyrate, and total SCFA were higher ($P < 0.05$) for wheat- compared with corn-based diets. On d 18, SB and WB inclusion increased

villi height (VH) ($P < 0.05$), and VH to crypt depth (CD) ratio (VH/CD), respectively ($P < 0.05$). On d 42 VH ($P < 0.05$) and VH/CD was higher in wheat-based diets ($P < 0.05$), whereas VH/CD was lower with SB supplementation ($P < 0.05$). There was significant three-way interaction for IDE on d 18 ($P < 0.05$). Wheat bran or SB had no influence on IDE in corn-based diets, whereas in wheat-based diets, WB inclusion tended ($P < 0.10$) to decrease IDE, but SB supplementation tended to improve ($P < 0.10$) IDE in wheat-based diets without WB, and significantly improved IDE in wheat-based diet with WB inclusion. Ileal DE was greater ($P < 0.05$) for wheat- compared with corn-based diets on d 42. In conclusion, SB supplementation in wheat or corn-based diets as well as with WB inclusion had the potential to improve digestibility and gut morphology.

Key Words: wheat bran; stimbiotic; short chain fatty acid; ileal digestible energy

127 Multicarbohydase and phytase complex supplementation improves performance and intestinal health of broilers fed DDGS diet. Gabriela Cardoso Dal Pont^{1*}, Cinthia Eyng³, Cristiano Bortoluzzi¹, Marcio Ceccantini⁴, Pierre Cozannet⁴, Michael Kogut², ¹Texas A&M, College Station, Texas, United States, ²USDA-ARS, College Station, Texas, United States, ³Unioeste, Marechal, Brazil, ⁴Adisseo, Antony, France.

Distillers dried grains and soluble (DDGS) have a high concentration of non-starch polysaccharides (NSP) mainly insoluble fiber. Variation in this amount of corn NSPs can impact the performance of broilers. Also, part of the DDGS's NSP can be broken down by exogenous enzymes producing prebiotic-like compounds. Thus, supplementation of enzymes in DDGS diets may allow the use of this ingredient for broilers, and improve intestinal health and performance. To test this hypothesis, a total of 288 day-of-hatch broilers were assigned to six treatments with eight replicates each. The treatments differed by the inclusion of DDGS in a corn-soybean based diet (0, 7 and 14%) and by the inclusion (on top) or not of a multicarbohydase (xylanase, arabinofuranosidase, and β -glucanase) and phytase complex (MCPC) using a completely randomized 3x2 factorial design (3 DDGS inclusion x 2 MCPC inclusion). Body weight gain (BWG), feed intake (FI), and feed conversion (FC) were calculated at days 7, 14, 21 and 28. Necropsies were performed at 14 and 28d, when small intestine was collected for "I See Inside" (ISI[®]) methodology (evaluation of histologic alterations of the intestine), where greater ISI[®] indicates worse intestinal health. Blood was collected at 7 and 28d for evaluation of intestinal permeability (FITC-d method) and intestinal inflammation (calprotectin biomarker). Data were submitted to analysis of variance considering the factorial arrangement and means from DDGS and MCPC groups compared ($P < 0.05$) by Tukey and F's test, respectively. No interaction was observed for BWG and FI, but MCPC supplementation increased BWG and FI at 7d. At 14d, the

inclusion of 14% of DDGS in the feed reduced BWG compared to 7% DDGS group, however, the supplementation of MCPC improved the BWG, FI, and FC of animals fed with 14% DDGS diet. The enzymatic complex improved the FI of birds in the 1-21d period. At 28d, MCPC supplementation improved FC of broilers fed with 7% DDGS and increased the FI and BWG in general. Broilers fed diets with MCPC presented a lower ISI[®] score (less alterations) in the duodenum and ileum at 14d. The MCPC reduced jejunum ISI[®] score of broilers fed with 14% DDGS diet at 14d. Additionally, supplementation of MCPC reduced ileal ISI[®] score of all groups at 28d. No effect of DDGS, MCPC or their interaction were observed for intestinal permeability and calprotectin. The present results indicate that the addition of a multicarbohydase and phytase complex allows an inclusion of 14% of DDGS without loss of performance and intestinal health compared to a corn-soybean based diet. Moreover, the observed benefits in performance might be related to the influence of the enzymes in the intestinal health.

Key Words: biomarker; DDGS; phytase; intestinal health; xylanase

128 Role of phytase and limestone particle size ratios on performance, egg quality and bone quality in post-peak phase of single cycled laying hens. Charis Waters^{1*}, Kelley Wamsley¹, Michael Elliot², Mike Bedford³, Craig Wyatt⁴, Pratima Adhikari¹, ¹Mississippi State University, Mississippi State, Mississippi, United States, ²A&E Nutrition Services, LLC, Lititz, Pennsylvania, United States, ³AB Vista Feed Ingredients, Marlborough, Wiltshire, United Kingdom, ⁴AB Vista Feed Ingredients, Plantation, Florida, United States.

The objective of this study was to investigate the role of the ratio of fine (F) and coarse (C) limestone and exogenous phytase at standard and superdosing levels on performance, egg quality and bone quality of post-peak Hy-Line W-36 hens from 40 to 60 weeks of age. A total of 560 hens (20 replicates of 4 hens per treatment) were randomly assigned into 7 experimental diets in a 2 x 3 + 1 factorial arrangement. The positive control (PC) was an industry standard that consisted of a 40F:60C limestone ratio without phytase and had 4.4% calcium (Ca) and 0.44% available phosphorus (avP). A common negative control (NC) diet was created by reducing 0.19% Ca and 0.17% avP of the PC. The factorial treatments consisted of two limestone ratios (40F:60C and 15F:85C) and three phytase levels (0, 400, and 1500 FTU) formulated from the NC. Hens were housed in A-frame cages with individual feeders at industry standard density, and feed was given at 100 g per bird per day. Data were analyzed using PROC GLM SAS 9.4. There was an interaction effect between phytase and limestone ratio with respect to hen day egg production (HDEP) ($P = 0.0022$), a significant increase in HDEP was observed in 15F:85C and 40F:60C with 400 FTU as compared to 15F:85C at 0 FTU and 40F:60C at 1500 FTU. Similarly,

there was a main effect of limestone ratio on feed intake (FI) ($P = 0.0236$), where we observed higher intake in 40F:60C groups compared to 15F:85C. We also observed a main effect of 400 FTU phytase level on feed conversion ratio (FCR) ($P = 0.0467$), where groups with 400 FTU had significantly lower FCR than 0 and 1500 FTU. Additionally, we observed a significantly higher albumen height ($P = 0.0013$) and Haugh unit (HU) ($P = 0.001$) in 400 FTU treatment groups as compared to 0 and 1500 FTU. In contrast, a significantly higher yolk weight ($P = 0.0299$) and lower albumen weight ($P = 0.0186$) were observed in 1500 FTU treatment groups compared to 400 FTU. Limestone ratios of 15F:85C had a significantly higher eggshell weight ($P = 0.0264$) as compared to 40:60. An interaction effect was observed between phytase and limestone ratio with tibia ash ($P = 0.0117$) in which we observed a significant increase in tibia ash with 40F:60C and 1500 FTU as compared to 40F:60C with 0 FTU. The study revealed that 400 FTU of phytase has benefits on various performance characteristics like HDEP and FCR compared with 0 and 1500 FTU. Also, 15F:85C groups showed a better effect on parameters like eggshell weight independent of phytase levels. Future studies could involve the digestibility of calcium and phosphorus due to added phytase levels as well as the breakdown of inositol levels in the gastrointestinal tract.

Key Words: bone quality; egg quality; laying hens; phytase; limestone particle size

129 Effects of phytase in a calcium and phosphorous-reduced diet on growth performance and bone mineralization of broilers challenged with *E. Maxima* and *E. Acervulina*. Hanyi Shi^{1*}, Taina Lopes¹, Yuguo Tompkins¹, Jinquan Wang¹, Janghan Choi¹, Woo Kim¹, ¹University of Georgia, Athens, Georgia, United States.

Phytase supplementation has been shown to improve growth performance and bone mineralization of chickens; however, there are limited literatures regarding dietary phytase and coccidia challenge in broilers fed with calcium (Ca) and phosphorous (P)-reduced diets. Therefore, an experiment was conducted to evaluate the effects of phytase in a Ca and P-reduced diet on growth performance and bone mineralization of broilers challenged with *Eimeria* spp. A total of 576 14-day-old male broilers were allocated to a 2 x 4 factorial arrangement with six replicates per treatment and 12 birds each. Two factors were coccidia challenge and four dietary treatments: 1) a positive control (PC; 0.84% Ca and 0.42% avP); 2) a negative control (NC; 0.74% Ca and 0.27% avP); 3) NC + 500 FTU/kg of phytase (NC500); 4) NC + 1500 FTU/kg of phytase (NC1500). Data were analyzed using SAS by two-way ANOVA via GLM procedure. $P < 0.05$ was considered significant, and means were further separated using Tukey's Test. During 0-6 and 0-9 D post-infection (DPI), coccidia challenge decreased ($P < 0.001$) BW, BWG, FI, fat percentage, fat weight, lean

weight, bone mineral density (BMD), bone mineral content (BMC), bone area, bone volume, fat free bone weight (FFBW), bone ash weight, bone ash percentage and bone ash concentration; and it showed a higher FCR ($P < 0.001$) compared to unchallenged group. Interaction effects of coccidial challenge and dietary treatments on FI and bone ash concentration were observed. From 0-9 DPI, the coccidia challenged groups showed lower ($P < 0.05$) FI compared to unchallenged groups, and the unchallenged NC500 showed a lower FI compared to unchallenged PC, whereas the challenged NC1500 group showed a higher FI compared to challenged PC. On 6 DPI, the unchallenged and challenged PC groups showed lower ($P < 0.05$) bone ash concentration compared to the unchallenged NC500 or

NC1500 group and the challenged NC1500 group. On both 6 and 9 DPI, Ca and P-reduced diet resulted in numerical increases on BMD, BMC, bone area, FFBW, bone ash weight, bone ash percentage and bone ash concentration compared to PC, and adding phytase at 500 or 1500 FTU/kg further increased ($P < 0.05$) these parameters. In conclusion, coccidial challenge compromised growth performance and bone mineralization on broilers, whereas Ca and P-reduced diet did not; and supplementing phytase improved bone mineralization regardless of coccidia challenge.

Key Words: bone mineralization; broiler; growth performance; phytase; coccidial challenge

Student Competition: Metabolism and Nutrition, Amino Acids

130 Effect of casein supplementation in corn diet on amino acid digestibility in corn and additivity of amino acid digestibility in corn and soybean meal for broilers. June Hyeok Yoon^{1*}, Changsu Kong¹, ¹*Kyungpook National University, Sangju-si, Gyeongsangbuk-do, Korea (the Republic of).*

The objectives of this study were to investigate the effect of casein supplementation in the corn diet on the ileal digestibility of amino acids (AA) for corn and additivity of apparent (AID) and standardized ileal digestibility (SID) of AA for the corn-soybean meal mixed diet (CSM) in broilers. A total of 168 day-old Ross 308 male broilers were fed commercial corn-soybean meal (SBM) mash diets during pre-starter (d 0 to 7), starter (d 7 to 21), and grower (d 21 to 31) phases. On day 31, birds were weighed and assigned in a randomized complete block design with 8 dietary treatments and 6 replicates. Four diets were included a nitrogen-free diet (NFD) and 3 diets containing 30, 60, or 90 g/kg of casein at the expense of cornstarch in NFD to determine the ileal digestibility of AA in casein by the regression method (4 birds/cage). Other 4 semi-purified diets consisted of corn diet, SBM diet, corn-casein mixed diet (CCM), or CSM (3 birds/cage). Corn, SBM, and casein were the sources of AA in experimental diets. All diets had 0.5% Cr₂O₃ as an indigestible index. On d 35, all birds were asphyxiated with CO₂ and then digesta from the distal two-thirds of the ileum was collected. Data were analyzed by one-way ANOVA using the GLM procedure of SAS. The REG procedure of SAS was used for the regression analysis. The ileal digestibility of AA in casein determined by the regression method ranged from 84.6% for Cys to 97.9% for Met. The AID and SID of AA for corn in the CCM were calculated by the difference method using the ileal digestibility of AA in casein derived from the regression method. The AID (Lys, Met, Phe, Val, Asp, and Ser) and SID (Arg, Lys, Met, Phe, Thr, Val, and Asp) for corn in the CCM was greater ($P < 0.05$) than those in the corn diet without casein. The predicted AID of all AA and SID of AA except for Cys in the CSM were not different from the measured AID and SID of AA irrespective of whether the digestibility of AA in corn was derived from the corn diet or CCM. In conclusion, the AID and SID of AA for corn increased with casein supplementation in the corn diet, and the AID and SID of AA for corn and SBM were additive regardless of whether the digestibility of AA in corn was derived from the corn diet or CCM for 35-day-old broilers.

Key Words: Broiler; Amino acid; Additivity; Basal ileal endogenous losses; Casein

131 Effects of commercial processing conditions on digestible amino acids in full fat soybean fed to broiler chickens. Ana Villegas^{1*}, Anita Menconi², Nadia Yacoubi², Todd Applegate¹, ¹*University of Georgia, Athens, Georgia,*

United States, ²*Evonik Operations GmbH, Hanau-Wolfgang, Germany.*

The objective of this experiment was to investigate the effect of varying extruder temperatures of full-fat soybean (FFSB) on apparent amino acid digestibility in broiler chickens. A batch of FFSB was same day processed in a commercial facility at extruder temperatures of 182, 199, and 154 °C to create three different soybean variants; control, overcooked and undercooked, respectively. The processed soybean batches were merged into three different diets at 35, 30, and 25% inclusion concentrations for the starter, grower, and finisher phases. All diets contained 0.5% titanium dioxide as an indigestible marker. A total of 1,860 male Cobb 500 broiler chicks were vaccinated with 1 x coccidia vaccine on d 1 via coarse spray and allocated to 3 treatment groups with 10 replicate floor pens (62 birds/pen) from 0 to 35 d of age. At d 14 and 28, birds were euthanized by carbon dioxide for the collection of ileal digesta. Both overcooked and undercooked processed soybean batches depressed the apparent ileal digestibility (AID) of all indispensable and dispensable amino acids at d 14 ($P < 0.05$) by 3.8% and 3.2%, respectively. Lysine represented the lowest decreased of AID among all indispensable amino acids for both; d 14 and 28. The AID of Lys was 7% lower ($P < 0.05$) in birds fed the overcooked soybean batch compared to the normal processed treatment at d14 and 28. These results demonstrated how suboptimal processing conditions influence amino acid metabolism of birds. Moreover, these findings stress the importance of optimizing a safe extrusion temperature range without penalty of the soybean nutrient value, which it has a meaningful impact on processing and live production costs.

Key Words: Coccidiosis; Amino Acid; Soybean; Apparent Ileal Digestibility

132 Effects of methionine levels in low or normal crude protein diets on the growth performance, body composition, gut health, and oocyst shedding on *Eimeria spp.* infected broilers. Guanchen Liu^{1*}, Venkata Choppa¹, Yuguo Tompkins¹, Woo Kim¹, ¹*University of Georgia, Athens, Georgia, United States.*

The present study aimed to investigate the effects of methionine levels in low or normal crude protein diets on growth performance, body composition, gut health, and oocyst shedding on *Eimeria spp.* infected broilers. A total of 600 male Cobb500 birds were challenged with 25,000 *E. maxima*, 25,000 *E. tenella*, and 125,000 *E. acervulina* sporulated oocysts on day 14. Birds were randomly assigned in a 5X2 factorial design (5 replicates/diet, 12 birds/replicate). The normal crude protein (NCP) diet contained 20% CP, and the low CP diet (LCP) contained 17% CP. Different levels of DL-methionine (Met) were supplemented in both diets to reach 0.52%,

0.70%, 0.85%, 1.00%, or 1.15% of total sulfur amino acids (TSAA). Growth performance was recorded on 6 and 9 days post-infection (DPI). On 9 DPI, the body composition was assessed by dual energy X-ray absorptiometry. Oocysts per gram (OPG) excreta were counted daily after infection. Two-way ANOVA, linear regression, and Duncan's multiple range test were used for data analyses. On 6 DPI, the increment of Met linearly decreased the feed intake in LCP ($P=0.03$) but not in NCP causing an interaction effect ($P=0.05$). Increased Met levels linearly increased the body weight and body weight gain (BWG) ($P<0.01$) on 6 DPI. The feed conversion ratio (FCR) linearly decreased as Met levels increased on 6 DPI ($P<0.01$) & 9 DPI ($P=0.04$); the linear relationship was found in both CP groups ($P<0.05$) on 6 DPI while only in NCP on 9 DPI ($P=0.03$). OPG of *E. acervulina* peaked on 6 DPI, and the *E. maxima* and *E. tenella* had peaked shedding on 6 & 7 DPI. On 6 DPI, Met level linearly increased OPG of *E. maxima* ($P=0.01$) and *E. acervulina* ($P=0.04$) in NCP. Moreover, in NCP, the 1.00% group had higher *E. acervulina* OPG than the 0.52% and 0.70% groups, and higher *E. tenella* OPG than the 0.52% group. On 7 DPI, the Met level linearly increased *E. acervulina* OPG ($P=0.03$) in LCP with higher OPG in the 1.15% group than in the 0.52% group. On 8 DPI, the 1.00% groups had higher *E. maxima* OPG than the 1.15% groups ($P=0.04$) while higher *E. acervulina* OPG than the 0.52% groups ($P<0.01$). OPG of *E. acervulina* in LCP were higher than NCP at 5 ($P=0.05$) & 8 DPI ($P<0.01$). However, the total OPGs of the three species were all higher in NCP than LCP ($P<0.05$) without TSAA effects. Linear TSAA% effects were found in bone mineral density (BMD) and content (BMC) ($P<0.05$). Especially in LCP, Met increment linearly decreased BMD and BMC ($P<0.05$). In conclusion, LCP diets seemed to have a suppressive effect on *Eimeria* reproduction. Increased Met supplementation would linearly improve the growth performance of *Eimeria*-infected birds. However, it might also favor *Eimeria* reproduction and decreased BMD and BMC.

Key Words: *Eimeria*; Broiler; Methionine; Coccidiosis; Total sulfur amino acid

133 Determining the optimal digestible isoleucine to lysine ratio of Ross 708 x Ross YP male broilers from 42 to 56 days of age. Dalton Dennehy^{1*}, Jordan Millican¹, Jason Lee², Roshan Adhikari², Kelley Wamsley¹, ¹Mississippi State University, Mississippi State, Mississippi, United States, ²CJ America Inc., Downers Grove, Illinois, United States.

The objective of this study was to reevaluate the optimal dIle:dLys ratio during the withdrawal phase (d 42-56). Ross 708 x Ross YP male chicks were provided common starter, grower and finisher diets (70, 69, and 69% dIle:dLys, respectively) formulated using breeder recommendations, as well as optimal dIle:dLys ratios established from our previous research. On d 42, pen weights were equalized by block (12 reps/trt), and each pen contained 25 birds (0.24

m²/bird); average BW was 3.206 kg. Experimental diets were created from a common deficient diet (54% dIle:dLys; 130% dLeu:dLys; 82% dVal:dLys). Crystalline Ile was added to the deficient diet to create the summit diet (84% dIle:dLys); intermediate levels of dIle:dLys (59-79% dIle:dLys) were obtained by blending these diets. A practical control diet (69% dIle:dLys; 146% dLeu:dLys; 78% dVal:dLys) was separately formulated and batched to verify blending techniques. This study was analyzed as a randomized complete block design using Proc GLM; significance was set at $P\leq 0.05$; LS means and calculated dIle:dLys ratios were utilized for regression analyses. Birds were individually weighed on d 55 to measure CV of BW and BW, d 42-55 BW gain, feed intake, and FCR. On d 56, 5 birds/pen of average weight (± 100 g) were processed, and yields were calculated. Metrics not significant included CV of BW, d 55 BW, and d 42-55 feed intake ($P>0.05$). Numerical and significant differences were observed for d 42-55 BWG and FCR ($P=0.0517$ and $P<0.0001$, respectively); FCR improved by 13 points as dIle:dLys increased from 54 to 84% (2.046 vs 1.921, respectively). Carcass and drumstick yields were significant, whereas feeding 54% dIle:dLys increased yields ($P<0.05$). Preplanned contrasts between the control diets demonstrated no differences for all metrics ($P>0.05$), verifying blending. Optimal dIle:dLys ratios were estimated using quadratic regression (QR; 95% of vertex), as well as linear and quadratic broken line models (LBL; QBL). The QR model estimated the ratio for BW at 72% ($P=0.0028$, $R^2=0.95$) and BWG at 71% ($P=0.0264$, $R^2=0.84$). For LBL, the ratio was estimated to be 65% for both BW ($P=0.0002$, $R^2=0.99$) and BWG ($P=0.0075$; $R^2=0.91$); QBL estimated the ratio at 70% for both BW ($P=0.0002$; $R^2=0.99$) and BWG ($P=0.0123$; $R^2=0.89$). For FCR, the QR model estimated the ratio to be 77% ($P=0.0125$; $R^2=0.89$), while LBL and QBL estimated it at 70% ($P=0.0171$; $R^2=0.87$) and 78% ($P=0.0118$; $R^2=0.90$), respectively. Overall, manipulating the dIle:dLys ratio had the largest impact on FCR, with a minor effects on BWG and processing. These data may be impacted by early nutritional strategies and other BCAA ratios, therefore future research should consider these factors when estimating optimal dIle:dLys.

Key Words: amino acid; broiler; isoleucine; withdrawal; optimal ratio

134 Evaluation of reduced crude protein diets supplemented with synthetic amino acids on egg production, egg quality, egg weight, and environmental impact of post-peak laying hens. Bryce Leopold^{1*}, Craig Coufal¹, Jason Lee², Roshan Adhikari², ¹Texas A&M University, College Station, Texas, United States, ²CJ America, Downers Grove, Illinois, United States.

The objective of this experiment was to evaluate the production, egg weight, egg quality, and environmental impacts of amino acid supplementation in reduced crude protein diets. The experimental design consisted of 5 dietary

treatments with 8 replicates per treatment and 9 birds per replicate for a total of 360 Hyline W-36 layers at 40 weeks of age. Diets were corn-soybean meal based and the treatments included a positive control (PC) with industry standard crude protein % supplemented with 3 amino acids (Lys, Met, Thr), negative control (NC) with 2% reduced crude protein supplemented with 3 amino acids (Lys, Met, Thr), and three additional treatments with 2%, 3%, and 4% crude protein reduction with all essential amino acids (Val, Ile, Arg, Trp) supplemented to reach the level of the PC. Average body weight (BW), egg production (%), egg weight (g), egg quality, feed consumption (FC), manure output (%), and mortality (%) were determined on weeks 40-59. Data was analysed via a one-way ANOVA with means being deemed significantly different at $P \leq 0.05$. The 2% reduction in crude protein in the NC decreased ($P < 0.05$) BW, % hen day egg production, egg weight, eggshell thickness, eggshell breaking strength, and manure output when compared to the PC. The 2% reduction in crude protein with amino acid supplementation yielded % hen day egg production, FC, BW, egg weight, eggshell thickness, eggshell breaking strength, and manure output to levels similar to that of the PC. The 3% reduction in crude protein with amino acid supplementation increased ($P < 0.05$) % hen day egg production while yielding FC, BW, egg weight, eggshell thickness, eggshell breaking strength, and manure output to levels similar to that of the PC. The 4% reduction in crude protein with amino acid supplementation decreased ($P < 0.05$) % hen day egg production, and egg weight when compared to the PC. Mortality, albumin weight %, yolk weight %, albumin solid %, and yolk solid % were not impacted by dietary treatments. These results suggest that a reduction in crude protein of an additional 2-3% beyond diets including Met, Lys, and Thr is achievable with amino acid supplementation to maintain equivalent essential amino acid ratios to maintain egg production and quality and reduced fecal output.

Key Words: amino acid; crude protein; egg production; egg quality; egg weight

135 Effects of different amino acid density diets on performance and carcass characteristics in broilers reared under thermoneutral, heat stress, and pair-feeding conditions. Jean-Rémi Teyssier^{1*}, Pierre Cozannet², Aurélie Preynat², Sami Dridi¹, Samuel Rochell¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Adisseo France S.A.S., Commeny, France.

In broilers, heat stress (HS) reduces feed intake (FI) and alters metabolism to ultimately reduce protein deposition

and growth. An experiment was conducted to determine if increasing dietary digestible amino acids (AA) could help minimize these effects. A total of 1,536 day-old Cobb male by-product chicks were reared under thermoneutral (TN) conditions until 20 d post-hatch before being allocated (12 birds/pen) into 8 houses divided into 16 pens. From 20 to 42 d, 4 houses were set at a TN temperature of 24°C, while the remaining 4 houses were set to 35°C to impart chronic HS. Additionally, 4 diets were formulated with 100% of recommended AA levels (CTL) or 135% of recommended levels of either all essential AA (AA+), Met, Arg, Thr (MRT), or Met, Arg, Thr, Ile, Val (MRTIV) and were distributed to 4 pens per house. In each TN house, birds from 8 pens were pair-fed (PF) to equalize FI of HS birds on the previous day, and birds from the remaining 8 pens were fed ad-libitum (AL). This resulted in 12 treatments and data were analyzed by ANOVA based on a split-plot design of environmental condition and dietary treatment. Performance was measured on 8 (AL and PF) or 16 (HS) replicate pens, while processing data were collected on 8 replicates per treatment with only half of the HS pens selected for those measurements. Over the challenge period, FI of HS birds (3.124 kg) was lower ($P \leq 0.001$) than that of AL birds (3.910 kg), and the BW gain (BWG) under HS (1.716 kg) and PF (1.882 kg) conditions were decreased ($P \leq 0.001$) compared to AL conditions (2.529 kg). Feed conversion ratio (FCR) was increased ($P \leq 0.001$) under HS (1.852 kg:kg) and PF (1.655 kg:kg) conditions compared to AL conditions (1.549 kg:kg). However, PF birds had higher ($P \leq 0.001$) BWG and lower ($P \leq 0.001$) FCR than HS birds. The FCR from 20 to 34 d was reduced ($P = 0.005$) with the AA+ (1.471 kg:kg) and MRT (1.480 kg:kg) diets compared to the CTL diet (1.447), regardless of environmental condition. Heat stressed birds also had lower ($P \leq 0.001$) total breast meat and higher chilled carcass ($P \leq 0.001$), wing ($P = 0.010$) and leg quarter (LQ) ($P \leq 0.001$) yields. When compared to HS birds, PF birds had lower chilled carcass and LQ yields. The AA+ diet decreased ($P = 0.008$) the fat pad yield under PF conditions compared to the CTL diet, and it increased ($P = 0.280$) chilled carcass yields independently of environment. Tender ($P = 0.034$) yield was also higher with the MRT diet, and all 3 increased AA diets increased ($P \leq 0.001$) LQ yield. Overall, increased AA density did not reduce the adverse effects of HS *per se* on performance or carcass characteristics, but increased density of all essential AA improved FCR and reduced the fat deposition of birds under both TN and HS conditions.

Key Words: Performance; Broiler; Heat stress; Amino acids; Carcass characteristics

Student Competition: Microbiology and Food Safety

136 Impact of cold storage and incubation of hatching eggs on the recovery of inoculated *Salmonella* Enteritidis or *Campylobacter coli* into yolk or albumen. Caitlin Harris^{1*}, L. Bartenfeld Josselson², Jeff Buhr², ¹University of Georgia, Athens, Georgia, United States, ²USDA-ARS USNPC, Athens, Georgia, United States.

Salmonella and *Campylobacter* are important foodborne pathogens and increased knowledge on the potential for egg transmission is beneficial when developing intervention strategies. Data is lacking for *Campylobacter* verifying vertical egg transmission but confirmed for *Salmonellae*. The objective was to evaluate recovery of *Salmonella* Enteritidis (SE; exp 1) or *Campylobacter coli* (CC; exp 2) when injected into albumen (AL) or yolk (YO) of hatching eggs that were cold-stored then incubated. For both exps, 240 SPF White Leghorn eggs were placed into 2 trt (n=120 AL and YO). On D0, 10³ CFU SE or CC inoculum was injected into albumen or yolk. Eggs were stored for 2d (6°C; 54%RH) and transferred to incubator for 15d (37.5°C; 55% RH). Fifteen eggs/trt were sampled on d0, storage days (S1 and S2), and days 1, 5, and 15 incubated (D1, D5, and D15). Total egg contents were sampled on d0, S1, S2, and D1. For D5 and D15, viable embryos were aseptically removed and sampled separately from egg contents. Direct and enriched plating was performed for egg contents/embryos in duplicate. Direct counts were analyzed with ANOVA and enrich results were analyzed with Kruskal-Wallis test to determine significance (p≤0.05). On d0, all samples were direct positive for exp 1 and 2. In exp 1, SE counts were similar for both S1 (0.9 Log₁₀ AL and YO) and S2 (0.9 Log₁₀ AL and 0.7 Log₁₀ YO). There was a significant difference in direct recovery of SE on E1 (0% AL vs. 100% YO); both were 100% following enrichment. SE recovery from egg contents on D5 and D15 were significantly different with YO 100% positive and AL 33 - 57% positive. For exp 1, no viable embryos were collected for YO and 1 viable embryo from AL was positive for SE on D5. In exp2, no significant difference in CC direct counts occurred on S1 (1.3 Log₁₀ AL vs. 1.8 Log₁₀ YO), but there was a significant difference in recovery on S2 (13% AL vs. 100% YO). YO had higher direct counts (0 vs. 1.0 Log₁₀) and enrich recovery (0% vs. 87%) on E1 compared to AL. For egg contents, there was a significant difference in recovery on E5 (0% AL vs. 100% YO), but no significant difference on E15 (0% AL vs. 9% YO). For YO, 11 viable embryos were sampled on E15, and there was a significant difference in enrich recovery (0% AL vs. 45% YO). The lack of viable embryos for exp 1 indicates that it is unlikely that SE contaminates the yolk of developing eggs, rather, it may be contaminating the vitelline membrane or albumen. In comparison, a small number of embryos survived to D15 of incubation when the yolks were injected with CC. Results confirm that AL/YO inoculation, cold storage for 2d, and

then incubation is a possible method to study vertical transmission of *Campylobacter* during incubation.

Key Words: *Campylobacter*; *Salmonella*; incubation; egg transmission; egg inoculation

137 Expression analysis of novel vaccine candidate genes of *Campylobacter jejuni* in a cell line infection study. Sabin Poudel¹, Linan Jia¹, Chuan-Yu Hsu², Pratima Adhikari¹, Aaron S. Kiess³, Li Zhang¹ ¹Department of Poultry Science, Mississippi State University, Mississippi State, Mississippi, United States, ²Institute for Genomics, Biocomputing, and Biotechnology, Mississippi State University, MS, Mississippi, United States, ³Prestage Department of Poultry Science, North Carolina State University, NC, North Carolina, United States.

Development of an effective vaccine will be a promising alternative to antibiotics to curb *Campylobacter jejuni* (*C. jejuni*) colonization in the gastro-intestinal tract of poultry to reduce the risk of human *Campylobacter* infection. In a previous study, utilizing a reverse vaccinology approach, we identified three novel conserved potential vaccine candidates suitable for the development of a vaccine. These three candidates [phospholipase A (*PldA*), TonB dependent vitamin B12 transporter (*BtuB*), and cytolethal distending toxin (*CdtB*)] are conserved among 206 analyzed *C. jejuni* strains. However, the expression of predicted genes during host-pathogen interaction is still unknown. The aim of this study was to analyze the expression levels of these candidate genes during the host-pathogen interaction. The gene expression levels were examined in four *C. jejuni* strains isolated from different sources (cloacal swab, retail chicken meat, bovine feces, and human clinical isolates). An avian macrophage-like immortalized cell line (HD11) was infected with these four *C. jejuni* strains. After infection, non-attached *C. jejuni* was removed, and total RNA was extracted from infected HD11 cells containing both prokaryotic and eukaryotic cells. Due to the limited and varied bacterial RNA contents among the infected host cells, pure bacterial RNA extracted from the bacterial culture was used as a control to generate a standard curve of 16S *rRNA* gene through serial dilution of cDNA to estimate the bacterial RNA content in treatment samples. The RT-qPCR assays were performed to determine the expression of the selected genes using gene-specific primers. The expression differences for each gene between control and treatments were analyzed with the $\Delta\Delta C_t$ methods using the *Campylobacter*-specific 16S rRNA gene as a reference for normalization. Fold change data were analyzed using an unpaired student's t-test, with a significant level set at $p \leq 0.05$. The *PldA* gene, which plays a major role in the hemolytic activity, was significantly upregulated from 3.41 to 65.41-fold higher than the negative control. The *BtuB* gene, an outer membrane vitamin B12 receptor, was upregulated from 4.03 to 44.07-fold higher than the

negative control. The *CdtB* gene, an extracellular protein responsible for producing toxins, was upregulated from 3.15 to 590.8-fold higher than the negative control. In conclusion, all three candidates were upregulated in four tested *C. jejuni* strains irrespective of their source of isolation. The upregulation of these genes during host-pathogen interaction indicates these genes might play a critical role during *C. jejuni* infection and colonization in chickens, making these genes the potential vaccine candidates.

Key Words: *Campylobacter jejuni*; gene expression; poultry; vaccine; RT-qPCR

138 Evaluation of diets supplemented with mannan oligosaccharides under a *Campylobacter jejuni* challenge model in broilers. Luis Munoz^{1*}, Marquisha Paul², James Krehling¹, Matthew Bailey¹, Kaicie Chasteen¹, Cesar Escobar Lobo¹, Yagya Adhikari¹, Maria Elliot¹, Kenneth Macklin¹, ¹Auburn University, Auburn, Alabama, United States, ²Alltech, Inc., Lexington, Kentucky, United States.

A study was conducted to assess the effects of dietary mannan oligosaccharides (MOS) with(out) a *Campylobacter jejuni* challenge on cecal colonization, broiler growth, carcass yield, and carcass contamination after processing. Two thousand two hundred- and forty-day-old Ross 708 males were randomly assigned within 8 experimental groups with a 4 x 2 factorial design, with 4 diets (negative control, positive control (bacitracin, 50 g/ton), MOS constant dose (400 g/ton), and MOS step-down dose (800, 400, and 200 g/ton in the starter, grower, and finisher periods, respectively) and with(out) a day 16 *Campylobacter jejuni* oral gavage challenge at 10³ CFU/mL dose. At day 24, 34, and 42 twenty-four birds per treatment were euthanized for cecal content collection for *C. jejuni* enumeration and prevalence calculation. On days 0, 14, 28, and 41 body weights and feed consumption were measured to determine broiler performance. At day 44, 80 birds per treatment were processed to determine carcass yield, followed by a wash of high-contact equipment in the processing line after each treatment to reduce risk of cross-contamination. Finally, on day 45, 16 carcass rinsates per treatment were collected for *C. jejuni*'s enumeration and prevalence calculation. All birds inoculated with *C. jejuni* had similar colony forming units (CFU)/ml in the cecal contents at days 24, 34 and 42 ($P > 0.05$) and all birds inoculated with phosphate buffered saline (PBS) were negative for *C. jejuni* after prevalence testing. The diet or type of inoculation did not influence growth performance ($P > 0.05$), however, the treatment supplemented with MOS and inoculated with *C. jejuni* had a trend to a lower feed conversion ratio in the starter and grower periods ($P = 0.0918$ and $P = 0.0550$, respectively). After processing: 1) carcass yield was similar in all treatments ($P > 0.05$), 2) *C. jejuni* inoculated birds with an unsupplemented diet had lower CFU/ml counts than the treatments supplemented with a positive control diet and the MOS constant dose diet

($P < 0.05$), and 3) all birds inoculated with PBS were negative for *Campylobacter*. Overall, the addition of MOS during a *C. jejuni* challenge did not have an impact on the reduction of *C. jejuni* colonization in the ceca, growth performance, carcass yield or carcass colonization after processing. Further experiments should evaluate the effects of MOS supplemented diets over intestinal morphology and immune system regulation.

Key Words: *Campylobacter jejuni*; Performance; Broiler; Colonization; Mannan Oligosaccharides

139 Disinfectant usage in barn cleaning alters the gut microbiome and increases carriage of *Campylobacter jejuni* in broiler chicken. Yi Fan^{1*}, Andrew Forgie¹, Tingting Ju¹, Camila Marcolla¹, Tom Inglis², Lynn McMullen¹, Benjamin Willing¹, Doug Korver¹, ¹University of Alberta, Edmonton, Alberta, Canada, ²The Institute of Applied Poultry Technologies, Airdrie, Alberta, Canada.

Biosecurity is important to maintain flock health in broiler chicken production. Currently, Canadian broiler chicken producers must deep clean their barns with chemical disinfectants at least once annually (full disinfection; FD) and may wash with water (water-wash; WW) between flocks throughout the year, though many choose FD between each flock. We hypothesized that FD would impact microbial functional capacity and inadvertently increase the likelihood of pathogen transmission. To test this, we conducted a cross-over experiment over four production cycles in seven commercial chicken barns to compare WW and FD focusing on the broiler performance, microbial composition, function and cecal short-chain fatty acids (SCFAs). Unpaired student's t test showed that the 30-day body weights ($p = 0.22$) and mortality rates ($p = 0.91$) were comparable between WW and FD. The 16S rRNA sequencing results suggested that the WW brought a modest but significant effect on the structure of broiler cecal microbiota (weighted-UniFrac; $\text{adonis } p < 0.05$), with significant reductions in *C. jejuni* abundance. In addition, the WW group showed increased cecal acetate, butyrate and total SCFA concentrations ($p < 0.05$), which were negatively correlated with *C. jejuni* abundance (Spearman's correlation coefficient test; $p < 0.05$). Shotgun metagenomics sequencing and functional analyses revealed that 564 genes and 47 pathways were differentially abundant between FD and WW chickens. Consistent with increased SCFA levels, several complex carbohydrate degradation-related and pyruvate fermentation pathways identified in *Helicobacter*, *Lachnoclostridium*, and *Megamonas* were more abundant in the WW group (empirical Bayes quasi-likelihood F-test, $\text{FDR-}p < 0.05$). The FD group showed enriched pathway including a fatty acid synthesis pathway harbored by *E. coli* ($\text{FDR-}p < 0.1$) and a UDP-N-acetyl-D-glucosamine biosynthesis pathway encoded by *C. jejuni* ($\text{FDR-}p < 0.1$). The results indicated that WW led to a gut microbiota with enhanced capacity for the production of SCFA, and reduced the risk of zoonotic

transmission of *C. jejuni* in broiler production relative to FD in the absence of a disease challenge.

Key Words: broiler chicken; *Campylobacter*; gut microbiome; short-chain fatty acid; barn disinfection

140 Trans-cinnamaldehyde nanoemulsions reduces *Salmonella* Enteritidis survival and trans-shell migration on eggs without affecting egg color or embryo development. Jodie Allen^{1*}, Brindhalakshmi Balasubramanian¹, Kimberly Rankin¹, Trushenkumar Shah Sr¹, Annie Donoghue², Indu Upadhyaya³, Yangchao Luo³, Abhinav Upadhyay¹, ¹University of Connecticut, Windsor, Connecticut, United States, ²University of Arkansas, Fayetteville, Arkansas, United States, ³University of Connecticut, Storrs, Connecticut, United States.

Salmonella Enteritidis (SE) is a major foodborne pathogen that causes enteric illnesses in humans, primarily through the consumption of contaminated poultry products. Generally Recognized as Safe phytochemicals such as trans-cinnamaldehyde (TC) have previously shown to exhibit anti-*Salmonella* efficacy, however, the low solubility of TC is a major hurdle in its adoption as an egg wash treatment. This study investigated the efficacy of TC nanoemulsions (TCNE) as wash treatments for reducing SE on eggs in the presence or absence of 5% chicken litter. In addition, the efficacy of dip treatments in reducing trans-shell migration of SE across shell barrier and effect on egg color and embryonic development of fertile eggs was investigated. TCNE were prepared by high-energy sonication using Tween-80 (Tw.80) or Gum Arabic and lecithin (GAL) as emulsifiers. White-shelled eggs were spot inoculated (200 μ L; attachment time 60 min) with a 4-strain mixture of SE (10^8 CFU/mL) followed by dipping in sterile deionized water (control) or water containing TC, or TCNE at 0.03, 0.06, 0.12, 0.24, 0.48% for 1, 3, or 5 min at 34°C. Post washing, the population of surviving SE on eggshell was enumerated on XLD agar. For trans-shell migration assay, following wash treatments, the egg internal contents were enriched in selenite cysteine broth, then incubated at 37°C for 48 h, followed by streaking on XLD agar for the presence of SE. All experiments had triplicate samples, repeated thrice, and analyzed using one-way ANOVA at $p < 0.05$. The nanoemulsions had a PDI below 0.3, particle size between 100-130 nm and zeta potential range of -6 to -30 mV. In baseline eggs (SE inoculated, not washed), a pathogen load of ~ 6.3 log CFU/egg was recovered. After washing with water for 5 min (control), ~ 4.4 log CFU/egg of SE were recovered. When washed with TC, only the highest concentrations (0.12, 0.24, 0.48%) were effective in reducing SE by $\sim 1.7, 2.8, 2.5$ log CFU/egg, respectively, as compared to control by 1 min ($P < 0.05$). TCNE-Tw.80 or GAL treatments (0.06, 0.12, 0.24, 0.48%) were effective in inactivating SE by at least 2 to 2.5 log CFU/egg as early as 1 min ($P < 0.05$). In presence of organic matter, 200 ppm chlorine (industry control), and TC were not effective in reducing SE on eggs ($P > 0.05$). However, nanoemulsions

(0.48%) reduced SE counts by ~ 2 to 2.5 log CFU/egg as early as 1 min ($P < 0.05$). Nanoemulsions also inhibited trans-shell migration of SE ($P < 0.05$) and shell color was not affected ($P > 0.05$). TCNE did not affect the growth of chicken embryo development by day 18 of incubation ($P > 0.05$). Results suggest that TCNE could potentially be used as an antimicrobial wash to reduce SE on eggs.

Key Words: *Salmonella*; eggs; Trans-cinnamaldehyde; nanoemulsions; food-grade emulsifiers

141 Effect of yeast-derived prebiotics on the cecal microbiota of roosters during *In Vitro* incubations. Jessica Brown^{1*}, Dana Dittoe¹, Doug Korver², Peter Rubinelli³, Si Hong Park⁴, Zachary Lawless³, Dale Thompson³, Steven Ricke¹, ¹University of Wisconsin - Madison, Madison, Wisconsin, United States, ²University of Alberta, Edmonton, Alberta, Canada, ³University of Arkansas, Fayetteville, Arkansas, United States, ⁴Oregon State University, Corvallis, Oregon, United States.

Prebiotics have the potential to improve gut health through modulating the gut microbiota. Therefore, the objective of this *in vitro* study was to evaluate the effects of commercial yeast-derived prebiotics (yeast product (YP) or yeast fermentate (YF)) on *Salmonella enterica* and the cecal microbiota of roosters. The feed only control (1) consisted of 20mL of anaerobic dilution solution (ADS) and basal diet (1.25% w/v). Cecal contents were obtained from 3, 53-wk old White Leghorn roosters fed a standard wheat-based, commercial basal diet, and diluted 1:3000 in ADS in an anaerobic chamber. Then, 20mL were aliquoted to separate cultures containing the following treatments: (2) ceca only; (3) basal diet; (4) basal diet+YP1; (5) basal diet+YP2 (6) basal diet+YP3; (7) basal diet+YP4; (8) basal diet+YF. Where applicable, basal diet was added to a final concentration of 1.25% w/v, prebiotics were applied at a rate of 1% w/v, and all cultures were inoculated with a nalidixic acid and novobiocin resistant strain of *Salmonella* Enteritidis (13A) at 10^7 CFU/mL. Cultures were incubated anaerobically at 37°C and sampled at 0, 24, and 48 h for *Salmonella* enumeration and at 48 h for microbiome sequencing (16S rDNA). Genomic DNA was extracted and resulting libraries were prepared and sequenced using an Illumina MiSeq. *Salmonella* data were log transformed and analyzed in a two-way ANOVA with means being separated using Tukey's HSD ($P \leq 0.05$). Sequencing data were analyzed in QIIME2 (2020.6) with diversity metrics (alpha and beta) and analysis of composition of microbiomes (ANCOM) being performed. Main effects were considered significant at $P \leq 0.05$ with pairwise differences being considered significant at $Q \leq 0.05$. There was an interaction of treatment and time on the load of *Salmonella* where treatments 4, 5, 7, and 8 reduced *Salmonella* by 1 log CFU/mL after 24 h compared to treatments 1, 2, 3, and 6. At 48 h, all prebiotic treatments reduced *Salmonella* by 3 log CFU/mL compared to the controls. There was no main effect of treatment on the alpha

diversity metrics, richness or evenness ($P > 0.05$). There was a main effect of treatment on the beta diversity, abundance and phylogenetic differences, but there were no pairwise differences ($P < 0.05$, $Q > 0.05$). Using ANCOM at the genus level, the abundance of the taxa *Synergistes*, *Alloprevotella*, *Sutterella*, and *Megasphaera* were significantly different ($W = 154, 147, 145, 140$, respectively). These results demonstrate the potential of these yeast derived prebiotics to reduce foodborne pathogens such as *Salmonella* Enteritidis, while not disrupting the indigenous microbiota. Further, genera such as *Synergistes*, a potential indicator of disease, was reduced in yeast-derived prebiotic treated cultures.

Key Words: *Salmonella*; Cecal Microbiota; Yeast Prebiotics; In vitro Incubation

142 The biofilm forming ability of *Salmonella* under fluidic shear stress on different surface materials. Hudson Thames^{1*}, Diksha Pokhrel¹, Emma Willis¹, Orion Rivers¹, Li Zhang¹, Thu Dinh¹, Wes Schilling¹, Shecoya White¹, Anuraj Sukumaran¹, ¹Mississippi State University, Mt Pleasant, South Carolina, United States.

One of the growing food safety concerns for the poultry industry is the persistence of *Salmonella* biofilms on processing surfaces. However, data on the factors influencing *Salmonella* biofilm formation are limited. The objective of this *in vitro* study was to characterize and quantify biofilm formation of various *Salmonella* strains (*S. Typhimurium* ATCC 14028, *S. Enteritidis* ATCC 4931, *S. Reading* turkey outbreak strain 0330 and reference strain 0326, and *S. Kentucky*) on four common processing plant surface materials (stainless steel, concrete, rubber, and polyethylene) under static and fluidic shear stress conditions using a crystal violet assay in conjunction with quantification. Surface material coupons were immersed individually in wells of a 24-well culture plate, which contained 1 mL of *Salmonella* culture ($6 \log \text{CFU/mL}$) in TSB or 1 mL of TSB as a control. The plates were incubated aerobically for 48 h at 37 °C either in static conditions or under shear stress (shaking at 150 rev/min). Biofilm density on the coupons was determined using a crystal violet assay by measuring the optical density (OD_{600}) and the biofilm cells were quantified by vortexing the coupons in TSB and plating on tryptic soy agar plates. Biofilms were also visualized using scanning electron microscopy. Data were analyzed by the GLIMMIX procedure of SAS 9.4 at a significance level of 0.05. On stainless steel, all strains formed a denser biofilm under fluidic shear stress than in static conditions (OD_{600} 0.08 and 0.04; $P < 0.001$). However, based on the OD_{600} -cutoff, all 5 strains formed weak biofilms under static conditions, whereas under shear stress, all strains formed moderate biofilms. On polyethylene, all strains formed denser biofilms under static conditions than under shear stress (OD_{600} 0.178 and 0.089; $P < 0.001$). However, despite these differences, the degree of biofilm formation did not change for four of the

five strains, based on the OD_{600} -cutoff. Enumeration revealed surface*incubation condition ($P = 0.024$) and surface*strain ($P < 0.001$) interactions in the number of biofilm-attached cells. Among all surface-incubation condition combinations, the highest number of biofilm-attached cells were found on polyethylene under fluidic shear stress ($6.4 \log \text{CFU/coupon}$; $P < 0.001$), irrespective of the strain. Biofilms of *S. Kentucky* on polyethylene had the highest number of cells ($7.8 \log \text{CFU/coupon}$) compared to all other strains-surfaces combinations, irrespective of the incubation conditions ($P < 0.001$). These results indicate that the biofilm formation of *Salmonella* is influenced by bacterial strain, surface material, and fluid movement on surfaces.

Key Words: biofilm; *Salmonella*; poultry processing; crystal violet; shear stress

143 Reduction of *Salmonella* Infantis using organic acids and surfactants alone and in combination. Andrea Urrutia^{1*}, Leticia Orellana Galindo¹, Marco Reina Antillon¹, Juan Figueroa¹, Alexandra Jackson¹, Kenneth Macklin¹, Dianna Bourassa¹, ¹Auburn University, Auburn, Alabama, United States.

Salmonella has been recognized as one of the most frequent causes of bacterial foodborne illness worldwide and is often associated with the consumption of poultry products. Pelargonic acid is a medium chain fatty acid, which has been previously shown to reduce *Salmonella* when applied to tomatoes. Lactic acid and peracetic acid are organic acids currently in use by the beef and poultry processing industries. Surfactants can be used to form an emulsion with organic acids to synergistically strengthen the antimicrobial activity. The objective of this study was to determine the antimicrobial activity of three different organic acids; pelargonic acid (PA), lactic acid (LA), and peracetic acid (PAA) alone and in combination with two different surfactants, Tween 80 or saponin, for the reduction of *Salmonella* Infantis. In 24 wells plates, PA concentrations of 300, 500, or 700 ppm, LA concentrations of 1,000, 2,000, or 5,000 ppm, and PAA at 75 or 135 ppm were evaluated alone and in combination with 0.05% of either Tween 80 or saponin. Each well was inoculated with 10 µl of a 10^5CFU/mL *Salmonella* Infantis inoculum and 250 µl of the treatments followed by a contact time of 5 minutes. Treatments were serially diluted and plated onto XLT4 agar plates for evaluation of *Salmonella* reductions. Count data were transformed to $\log_{10} \text{CFU/mL}$ and reductions analyzed using the GLM procedure with means separated by Tukeys HSD with significance at $P \leq 0.05$. PA did not reduce *Salmonella* Infantis when applied alone or in combination with Tween 80. However, when combined with saponin, PA reduced *Salmonella* below the level of detection ($< 100 \text{CFU/mL}$, $P < 0.0001$). LA at 2,000 alone and in combination with Tween80 and at 5,000 ppm reduced *Salmonella* below the level of detection ($P < 0.0001$). Combinations of LA with surfactants did not

improve antimicrobial activity. PAA at 75 and 135 ppm both reduced *Salmonella* below the level of detection ($P < 0.0001$). Neither saponin (0.09 log₁₀ CFU/mL reduction) nor Tween 80 (0.08 log₁₀ CFU/mL reduction) alone were effective at reducing *Salmonella* Infantis. Based on the results of this study, PA has potential for use as an antimicrobial for the reduction of *Salmonella* Infantis in poultry when used in combination with the surfactant, saponin.

Key Words: Salmonella; saponin; lactic acid; peroxyacetic acid; pelargonic acid

144 High-pressure processing reduces *Salmonella* and *Campylobacter* in chicken parts. Sasikala Vaddu^{1*}, Manpreet Singh¹, Harshavardhan Thippareddi¹, ¹University of Georgia, Athens, Georgia, United States.

High Pressure Processing (HPP) also known as cold pasteurization is a non-thermal food and beverage preservation method. It is based on the use of high isostatic pressure transmitted by the water of up to 6,000 bar /600MPa /87,000 psi, held for a few minutes. HPP has been used to improve shelf life and microbiological safety of a variety of meat and non-meat food products. The objective of this study was to evaluate the efficacy of HPP against *Salmonella* and *Campylobacter* in chicken parts (breast, wings and skin). Fresh chicken breast fillets, wing flats and skin were obtained from a commercial processor. Breast fillet was sliced to cubes of 2.5 cm, and skin was cut to squares of 2.5 cm while wing flats were used as whole. The products were inoculated with a cocktail of nalidixic acid-resistant *Salmonella* Typhimurium (ST^N; ca. 6 log₁₀ CFU/mL) and gentamicin resistant *Campylobacter coli* (CC^G; ca. 5 log₁₀ CFU/mL). The inoculum was allowed to attach for 15 min, products were vacuum-packed in high barrier bags and cooled to 4°C. The products were subjected to HPP (85 MPa) for 1 s, 1.25, 2.5 or 5 min. HPP treated samples were minced in chilled buffered peptone water (100 mL), serially diluted in peptone water, and spread plated on Brilliant Green Sulfa agar supplemented with nalidixic acid (200 ppm) for enumerating *Salmonella* and on Campy Cefex agar containing gentamicin (200 ppm) to enumerate *Campylobacter*. Three independent replications (n=3) were performed using freshly obtained chicken parts on three different days. Data were analyzed using one-way ANOVA in the GLM of SAS 9.4 (2004; SAS Institute Inc., Cary, NC). Tukey's test was used to separate the means of the microbial reductions (log₁₀ CFU/mL) and significant difference at $p \leq 0.05$ was used. HPP resulted in ST^N reductions ($p \leq 0.05$) of 5.90, 5.01 and 6.09 log CFU/mL within 5 min in chicken breast, wings and skin, respectively. CC^G population reductions ($p \leq 0.05$) of >5.00 log CFU/mL were observed within 1 s of HPP treatment in all the chicken parts. *Campylobacter* was more sensitive to HPP treatment compared to *Salmonella* in chicken parts. HPP of chicken parts (breast, wings and skin) can improve microbiological safety of the products by reducing *Salmonella* and

Campylobacter populations. Meat quality of breast meat should be evaluated prior to application of HPP to chicken breast fillets.

Key Words: Campylobacter; Salmonella; wings ; high-pressure processing; chicken parts

145 Buffering capacity comparison of tris-phosphate-carbonate (TPC) and buffered peptone water (BPW) *Salmonella* pre-enrichments for manufactured feed and feed ingredients. Cesar Escobar Lobo^{1*}, Luis Munoz¹, Matthew Bailey¹, James Krehling¹, Wilmer Pacheco¹, Ruediger Hauck¹, Jeff Buhr², Kenneth Macklin¹, ¹Auburn University, Auburn, Alabama, United States, ²USDA ARS, Athens, Georgia, United States.

Various comprehensive culture-based methods to detect *Salmonella* in animal feed have been developed due to the impact of this bacterium on public and animal health; these methods generally include pre-enrichment (non-selective medium), enrichment (selective medium), sample isolation and serological confirmation. Recovery of *Salmonella* depends on the type of sample, level of stress/injury on the bacteria, presence of competitive bacteria, and level of *Salmonella* contamination. A pH 6.5 is near the ideal pH range for *Salmonella* growth and pH values below 5 could affect detection and recovery. Tris-phosphate-carbonate (TPC) is a new pre-enrichment buffer that demonstrated a strong buffering capacity. For this project, buffered peptone water (BPW) and TPC buffering capacity were evaluated as pre-enrichment mediums for detection of *Salmonella* in animal feed and feed ingredients. A total of 185 samples (feed ingredients and finished feed) were collected from 5 commercial feed mills (A, B, C, D and E), 2.5 g of sample were mixed with 22.5 mL of pre-enrichment and incubated, pH was measured at 0 and 24 h. Data was analyzed using one-way ANOVA (GLM) (significant $P \leq 0.05$), means were separated using Tukey's HSD and pH change in time (initial-final) was analyzed using independent *t*-test for each sample type in SPSS® software. Differences were observed in pH values ($P < 0.001$), post mixing samples of feed mill A and ground corn of feed mill C reported higher initial pH value (0 h) for TPC (8.29) and BPW (7.24) respectively. Meat & bone meal of feed mill E reported higher final pH value (24 h) for TPC (7.39) and ground corn of feed mill A for final BPW (6.64). Pellet loadout of feed mill E reported lower pH value for final TPC (6.18) and final BPW (4.81). Moreover, differences were observed comparing pH values by type of sample ($P < 0.001$), Corn DDGs samples presented lower initial pH values for TPC (6.97) and BPW (5.75), while pellet loadout presented lower final pH values for TPC (6.26) and BPW (5.12). Additionally, using *t*-test, ground corn and mash loadout samples reported differences in pH change ($P < 0.002$) for both, BPW and TPC. Pellet loadout (21.6%) and wheat midds (27.4%) experienced the greatest pH change at 24 h using BPW, whereas pellet loadout (21.6%) and post cooling (20.2%) using TPC. From meat &

bone meal samples, 4 were positive for *Salmonella*, 3 in BPW and 1 in TPC; for the same samples, initial-final pH for BPW was 6.72-6.39, 7.23-6.28, 7.21-6.44 and for TPC 7.81-7.43. Results show the buffer capacity of two-pre-enrichments and how some feed ingredients and finished feeds change pH during incubation. Samples from additional feed mills will be collected and analyzed to complete this project.

Key Words: *Salmonella*; pre-enrichment; buffer; BPW; TPC

146 Bile salt hydrolase improves taurodeoxycholic acid against *Clostridium perfringens* growth. Tahrir Alenezi^{1*}, Ying Fu¹, Ayidh Almansour¹, Mohit Bansal¹, Hong Wang¹, Xiaolun Sun¹, ¹*University of Arkansas, Fayetteville, Arkansas, United States.*

Clostridium perfringens is the main pathogen of chicken necrotic enteritis (NE) with huge economic loss in the poultry industry. Secondary bile acid deoxycholic acid (DCA) in feed reduces chicken NE but the accumulation of DCA in the gut is conjugated tauro-DCA (TDCA). The gut microbiota catalyzed deconjugation bile acids using bile salt hydrolase (Bsh). We hypothesized that deconjugating TDCA by Bsh would improve dietary DCA efficacy against NE. *C. perfringens* growth inhibition assay was conducted with bile acids tauro-cholic acid (TCA), CA, TDCA or DCA. The tryptic soy agar (TSA) plates supplemented with 0.2% TDCA and 0.03% CaCl₂ were used to examine the Bsh activity. The *bsh* genes were PCR-amplified, cloned into plasmids pET-28a (pET-Bsh) and pDR111 (pDR-Bsh), and Sanger-sequenced. After transformed pET-Bsh plasmid into *E. coli* DH5a and BL21 for amplification and protein expression, respectively, the His-tag purified Bsh was evaluated by SDS-PAGE, Dot-Blot, DCA plate precipitation, and *C. perfringens* inhibition assay. pDR-Bsh transformed into *Bacillus subtilis* was assessed by *C. perfringens* inhibition assay. Notably, the inhibition of *C. perfringens* growth by bile acids was in the order of DCA>TDCA>CA>TCA. *Bifidobacterium longum* in anaerobic air secreted Bsh showed as colonies surrounded by opaque precipitation zones on the TSA plates with TDCA and CaCl₂. After cloned, transformed, and expressed pET-Bsh in BL21, Bsh protein was His-tag purified, subjected to SDS-PAGE, and showed an expected band of 32 kDa. Bsh expression was also verified by Dot Blot assay using anti-His-tag antibody. Bsh activity was then examined on the TSA plates, showing opaque crystallized DCA in the path of Bsh solution compared to a transparent path from the elution buffer only. *C. perfringens* growth inhibition assay showed that TDCA reduced the bacterium growth in the presence of purified Bsh or supernatant of *B. subtilis* transformed with pDR-Bsh. In conclusion, DCA was more potent than other bile acids against *C. perfringens* growth. Bsh cloned from *B. longum* to *E. coli* or *B. subtilis* was able to inhibit *C.*

perfringens growth in the presence of TDCA, suggesting potential new intervention against chicken NE.

Key Words: necrotic enteritis; Bile salt hydrolase; conjugated bile acids; *C. perfringens* inhibition assay

147 Microbiological assessment of poultry feed through the pelleting process using different conditioning temperatures. Marco Reina Antillon^{1*}, Juan Figueroa¹, Alexandra Jackson¹, Cesar Escobar Lobo¹, Jose Vargas¹, Orlando Fiallos¹, Wilmer Pacheco¹, Dianna Bourassa¹, ¹*Auburn University, Auburn, Alabama, United States.*

The objective of this study was to evaluate the effect of conditioning temperature (80, 85, and 90°C) on microbial load reduction during conditioning and pelleting. A corn-soybean meal-based basal diet was produced using a twin shaft mixer and then conditioned at 80, 85 and 90°C, maintaining a retention time of 40 seconds and pelleted through a 4.0-mm pellet die using a pellet mill at a constant production rate of 636 kg/h. Samples were collected every 30 seconds after mixer, conditioning, pelleting, and cooling. The presence of *Salmonella*, Aerobic Plate Count (APC), *E. coli* and coliform counts were evaluated. All samples were suspended in buffered peptone water, then serially diluted, and plated for bacterial counts. For *Salmonella* evaluation, samples were enriched, plated, and all suspect colonies were examined. Data was analyzed as one-way ANOVA and means were separated using Tukey's HSD with statistical significance considered at $P \leq 0.05$. None of the samples were positive for *E. coli* or *Salmonella*. The conditioning temperature did not influence APC or coliform counts ($P=0.1050$, $P=0.6581$, respectively), possibly attributed to the short retention time and exposure to steam in the conditioner. Nevertheless, significance was found among the sampling points. Mixer samples had higher ($P=0.0077$) APC compared to samples collected after conditioner, pelleting, and cooling (5.84 vs. 3.93, 4.26, and 4.47 Log₁₀ CFU/g). The thermal process during conditioning led to a reduction of ~ 2 Log₁₀ CFU/g. Mixer samples had the highest coliform counts (3.85, $P<0.0001$) which decreased to 0.63 Log₁₀ CFU/g following conditioning, then increased following pelleting (0.80) and cooling (1.79 Log₁₀ CFU/g). Conditioning samples indicate a reduction of ~ 3 Log₁₀ CFU/g. However, samples after cooling had an increase of ~ 1 Log₁₀ CFU/g, likely attributed to the mechanism of cooling used for this experiment (ambient air). Overall, the conditioning and pelleting process reduced APC and coliform counts.

Key Words: feed; pelleting; poultry; coliforms; microbiology

148 Microbiological survey of wastewater and solids from poultry processing plants after dissolved air flotation (DAF) treatment. Marco Reina Antillon¹, Wellington Arthur^{1*}, Leticia Orellana Galindo¹, Andrea Urrutia¹, Alexandra Jackson¹, Steve Kitchens¹, Kenneth

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Poultry Processing Wastewater (PPW) and the solids generated after dissolved air flotation (DAF) treatment are rich in organic matter which makes them a potential source of nutrients for crop production. The suitability for irrigation or land application depends on physicochemical and microbiological characteristics. The objective of this study was to survey PPW and DAF solids to determine microbial load and the presence of foodborne pathogens. PPW and DAF solids were collected from eight broiler processing plants. Aerobic Plate Count (APC), *Escherichia coli* and other coliforms, and the presence of *Salmonella* and *Campylobacter* were evaluated. For the enumeration of bacteria, water samples were serially diluted and plated on appropriate petrifilms. Solids were suspended in buffered peptone water, serially diluted, and plated. For *Salmonella* and *Campylobacter* evaluation, samples were enriched, plated, and presumptive colonies were confirmed. For each positive *Salmonella* sample, isolates were evaluated to determine serotype. For PPW, APC ranged from 2.8 to 7.5 log₁₀ CFU/mL, total coliforms ranged from 0.4 to 6.8 log₁₀ CFU/mL, and *E. coli* ranged from 0 to 4.5 log₁₀ CFU/mL. The presence of *Salmonella* and *Campylobacter* in PPW was detected in five plants and two plants, respectively. For solids, APC ranged from 2.5 to 8.6 log₁₀ CFU/g, total coliforms ranged from 3.7 to 6.9 log₁₀ CFU/g, and *E. coli* ranged from 0 to 5.6 log₁₀ CFU/g. *Salmonella* was detected in the solids from seven plants, but *Campylobacter* was only present in one plant. The *Salmonella* serotypes identified include Cerro, Enteritidis, Indikan, Infantis, Kentucky, Mbandaka, Newport, Rough_O:r:1,5, Tennessee, Uganda_var_15+, and 34+. Although PPW and solids are a potential source of nutrients, pathogens may need to be mitigated before use for crop irrigation or land application.

Key Words: Foodborne pathogens; Food safety; Poultry processing wastewater; Microbiology

149 Effects of a turkey-origin *Lactobacillus salivarius* probiotic and a live attenuated *Salmonella* Typhimurium vaccine on the cecal colonization and internal organ dissemination of emerging multidrug-resistant *Salmonella* in growing turkeys. Shijinaraj Manjankattil^{1*}, Grace Dewi¹, Claire Peichel¹, Medora Creek¹, Peter Bina¹, Kay Lerohl¹, Kerem Deniz¹, Mastura Akhtar¹, Robert Porter¹, Timothy Johnson¹, Sally Noll¹, Anup Kollanoor Johny¹, ¹University of Minnesota, Saint Paul, Minnesota, United States.

The emergence of uncommon *Salmonella* serotypes with the potential to cause foodborne outbreaks linked to turkeys

demands sustainable broad-spectrum *anti-Salmonella* approaches. We investigated the effects of two preharvest interventions [turkey-origin *Lactobacillus salivarius* and a live, attenuated *S. Typhimurium* vaccine] against a cluster of three emerging turkey-sourced *Salmonella* serotypes (*S. Reading*, *S. Saintpaul*, *S. Agona*) in 6-week-old grower turkeys. Two experiments were conducted. In each experiment, thirty, day-old turkey poults were randomly distributed into five treatment groups, two control groups [Negative control (NC) and Positive control (PC) groups], an *L. salivarius* (LS) group, a vaccine (VC) group, and a combination (LSVC – *L. salivarius*+vaccine) group. The NC and PC groups did not receive the probiotic or vaccine. The LS and LSVC groups received *L. salivarius* through drinking water from day 1 until the end of the study (10⁵ CFU/ml). According to the manufacturer's recommendation, birds belonging to VC and LSVC groups received the vaccine (AviPro Megan Egg). At week 5, all birds except the NC group were inoculated with 6 log₁₀ CFU/bird *Salmonella* (containing a three-serotype mixture of *S. Agona*, *S. Saintpaul*, and *S. Reading*) by crop gavage. Birds were euthanized and necropsied for sample collection on day 7 after inoculation. Cecal samples collected in 10 mL phosphate buffer saline were weighed, homogenized, and serially diluted in PBS. Appropriate dilutions were plated on xylose lysine deoxycholate with nalidixic acid (XLD+NA) agar plates and incubated at 37°C for 24 hours. Liver and spleen samples were enriched at 37°C for 24 hours and streaked on XLD+NA plates. Liver samples were subjected to histopathology and immunohistochemistry analyses. Statistical analysis was done using the PROC-MIXED procedures of SAS, and a significant difference was determined at *P*<0.05. The LS and VC groups resulted in 1.7 and 1.5 log₁₀ CFU/g reductions of *Salmonella* in the cecum of turkeys compared to the control (*P*< 0.05). The combination group, LSVC, resulted in a 1.6 log₁₀ CFU/g *Salmonella* reduction compared to the controls (*P*<0.05). LSVC resulted in 92% of *Salmonella*-negative liver and spleen samples compared to the control group. However, LS and VC alone resulted in 50% and 42% *Salmonella*-negative liver samples and 42% and 42% *Salmonella*-negative spleen samples, respectively. No pathological differences were apparent between the treatment groups. This study indicates the potential of turkey-derived *L. salivarius* and the *S. Typhimurium* vaccine to reduce the propagation of emerging serotypes of *Salmonella* when multiple serotypes colonize turkeys (MTRPC#2019-02).

Key Words: turkeys; *Lactobacillus*; Food safety; *Salmonella* Vaccine; Preharvest

Student Competition: Metabolism and Nutrition, Vitamins and Minerals

150 Breeders fed diets supplemented with conventional free or lipid microencapsulated premix forms of trace minerals at standard or high levels.

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Numerous poultry feeding trials have been conducted to determine the effects of trace mineral (TM) dietary supplementation on live performance, egg and meat quality. Nevertheless, TM requirements for poultry have not been fully defined, with considerable variation in recommendation levels for poultry sectors and feeding phases. Lipid matrix microencapsulation of TM (EDDI, Ferrous Sulfate, Sodium Selenate, Zinc Oxide, Manganese Oxide, Copper Sulfate) at normal and excessive levels is hypothesized to improve production performance of broiler breeder when compared with normal and excessive levels of inorganic TM salts. Therefore, we aimed to determine the effects of TM form supplemented in poultry diets on body parameters, number and quality of the eggs produced. At 27 weeks of age, 108 Aviagen broiler breeder females (Ross 708) and 24 males (Ross HY) were randomly distributed among 12 pens with 9 females and 2 males per floor pen in an environmentally controlled room. Each pen was assigned to one of 4 dietary treatments consisting of a factorial arrangement of 2 mineral premix forms (free and microencapsulated) and 2 mineral premix supplement dosages (100% and 200% of Aviagen recommendations): 1) Free 100% (FR100); 2) Microencapsulated 100% (MI100); 3) Free 200% (FR200); and 4) Microencapsulated 200% (MI200). Broiler breeder performance characteristics were tabulated from 27 to 55 weeks of age and summarized weekly. At 54 weeks of age, ~200 eggs from each treatment were incubated in order to assess fertility. At termination (55 weeks), 15 hens per treatment were randomly selected to access morphology of the jejunum mucosa and tibia bone parameters. The breeder hens that received the microencapsulated trace minerals (MI) showed improved egg production on a percent Hen-Day, and Hen-Housed basis, along with feed conversion (kg feed/dozen eggs) ($P < 0.05$). There were no significant differences observed in the histology parameters with regards to form of TM supplemented, but hens fed TM 100 dose presented with greater villus height, crypt depth, and villi area than hens fed TM 200 dose ($P < 0.05$). There were no significant treatment effects on bone weight, length, and bone mass density (BMD). However, microencapsulated treatments increased tibia bone thickness, and free trace mineral supplementation increased ash% and Cu content of tibias, regardless of premix form. Hens fed TM 200 had higher bone Mn content than TM 100, whereas hens fed MI100 had the highest Fe content. Feeding lipid matrix microencapsulated trace minerals to broiler breeders may be

more beneficial to enhance egg production parameters and number of chicks produced per hen than feeding breeders with conventional free-form trace mineral premix.

Key Words: Nutrition; Mineral; Broiler Breeder; Microencapsulation; Lipid Matrix

151 Effect of dietary organic chromium on growth performance, blood metabolites, and myopathies in broilers subjected to heat stress.

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Supplementation with organic chromium (Cr) in broiler diets has a beneficial role in the productive and reproductive performance, reducing negative effects due heat stress. A completely randomized experiment was conducted where the objective was to evaluate organic Cr supplementation in broiler diets and its effect on growth performance, blood and hormonal parameters, and meat quality and composition. One-d-old chicks ($n = 1,000$; Cobb 500) were assigned to 10 replicate pens (20 birds per pen) and fed 1 of 5 dietary treatments (0, 0.13, 0.25, 0.50, and 1.0 mg Cr per kg of feed). Birds were reared for 21 d under normal temperature according to breeder recommendations and then subjected to cyclic heat stress (34 °C) from 0900 to 1500 h on d 22 to 42. Growth performance was determined on d 21 and 42. At d 41, 1 bird per pen was randomly selected for blood collection. Plasma samples were analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), glucose (GLU), lactate dehydrogenase (LDH), total protein (TP), triglycerides (TRI) and creatine phosphokinase (CPK) with a high-performance automatic spectrophotometer. Corticosterone (COR) and thyroxine (T₄) were quantified using a monochromator-based microplate reader. At d 42, 4 birds per pen were euthanized and breast muscles were used to evaluate: Wooden Breast (WB) and White Striping (WS) myopathies. A portable colorimeter was used for analysis of meat color for lightness (L*), redness (a*), and yellowness (b*) at 15 min and 24 h post-mortem expressed in the CIE Lab color space. Thiobarbituric acid reactive substances (TBARS) analysis was performed for lipid peroxidation after 10, 30, and 60 d of storage at -20 °C. Data were analyzed as a 1-way ANOVA using SAS PROC GLM with orthogonal polynomials and a regression analysis was performed when a linear or quadratic effect with a 95% confidence interval was observed. No differences were observed among treatments for growth performance from d 0 to 21 and d 22 to 42 ($P \leq 0.0646$). For plasma analysis, a decreasing linear effect was observed for TRI ($P = 0.048$) and there were no

effects on any other variables evaluated. Treatments did not alter WB ($P = 0.9326$) and WS ($P = 0.5574$). For color analysis 15 min post-mortem, a quadratic effect was observed for A*, the maximum redness observed was 2.10 ($P = 0.0064$), and a linear increase in lightness (L*; $P = 0.0027$). A quadratic effect was observed for TBARS after 60 d of storage, with the maximum concentration of 0.063 mg of malondialdehyde per kg of meat ($P = 0.041$). In conclusion, organic Cr supplementation did not affect growth performance, COR, T4, or incidence and severity of WB or WS in heat stressed broilers.

Key Words: antioxidant; broilers; White Striping; Wooden Breast; organic chromium

152 Evaluation of five commercial limestones on laying hen performance, bone ash, and total tract calcium retention. Rebekah Drysdale^{1*}, Pam Utterback¹, Jennifer Blair¹, Benjamin Parsons¹, Minoy Cristobal¹, Kersten Bornholt¹, Elizabeth Kim², Janet Remus², Carl Parsons¹, ¹*University of Illinois Urbana-Champaign, Urbana, Illinois, United States*, ²*Danisco Animal Nutrition & Health/IFF, Wilmington, Delaware, United States*.

The objective of this study was to determine the effect of feeding five different commercial limestones varying in solubility (88-97%) on laying hen performance, eggshell quality, and total tract calcium retention when the limestones were fed at two different dietary calcium levels. Lohmann LS Lite laying hens were fed one of 10 diets from 50 to 66 weeks of age. All diets were corn-soybean meal based and contained 16% protein. The 10 dietary treatments were arranged in a 5 x 2 factorial design with five limestones (L1-L5) and two dietary calcium levels which were 3.80 and 2.65%, respectively. Each of the 10 diets were fed to six replicate groups of 14 caged hens. The Ca solubilities for the limestones (L1-L5) were 97, 88, 95, 97, and 93%, respectively. Egg production, egg weight, daily egg mass, feed consumption, feed efficiency, egg specific gravity, eggshell breaking strength, tibia bone ash, and total tract calcium retention were measured for each of the 60 replicate groups of hens at 2 or 4 week intervals. Data were analyzed using a two-way ANOVA for a 5 x 2 factorial design. There was a significant ($P < 0.05$) main effect for dietary calcium level for hen-day egg production, egg mass, feed efficiency, egg specific gravity, and eggshell breaking strength with the values being decreased for hens fed the lower 2.65% calcium level. When comparing the five limestones, L1 generally yielded the highest performance values and was selected as the primary reference limestone for comparison with the other limestones. Hen-day egg production was significantly lower ($P < 0.05$) for hens fed diets containing L2, L3, or L5 compared with hens fed L1 in the lower calcium diets. Egg mass for hens fed L2 was decreased compared with hens fed L1 in the lower calcium diet. Feed efficiency for hens fed L2 was lower ($P < 0.05$) than that for hens fed L1 at both dietary calcium levels. No effect of limestone source was observed for feed

consumption, egg weight, egg specific gravity, and eggshell breaking strength except that hens fed L2 had significantly lower ($P < 0.05$) egg specific gravity and eggshell breaking strength than hens fed L3 at the higher calcium level. There was no significant effect of dietary treatments on bone ash expressed as g/tibia or as %. There was also no consistent effect of limestone source on total tract calcium retention and values were higher ($P < 0.05$) at the lower dietary calcium level. The results of this study indicated that the L2 limestone source had the lowest solubility (88%) and was generally inferior to the other four commercial limestones primarily based on responses for egg production, egg mass, and feed efficiency.

Key Words: bone ash; calcium; laying hen performance; limestone; calcium retention

153 Effects of age of lay, egg storage length, and level of non-phytate phosphorous on egg quality parameters and mineral contents of eggs from 24- and 32-week-old laying hens fed corn-soybean meal-based diets. Malia Jones^{1*}, Michael Ford¹, Jacqueline Jacob¹, Anthony Pescatore¹, Sunday Adedokun¹, ¹*University of Kentucky, Lexington, Kentucky, United States*.

This study investigated the effects of age at lay, storage length, and level of non-phytate phosphorous (NPP) on egg quality and mineral contents of eggs in a randomized complete block design. A total of 252 hens were fed corn-soybean meal-based diets containing 3 levels of NPP (90, 100, and 110% of the requirement; 0.51% per Hyline Management Guide) with 7 replicates of 12 birds. Two sets of eggs per replicate were randomly collected at wk 24 and 32 of age. One set of eggs (6 per replicate) was analyzed on day 0 and the other set was weighed and stored at 4°C for 45 days. The contents of two eggs per replicate from 24 wk-old hens were analyzed for minerals (Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn). Egg quality evaluated included yolk color, yolk, albumen, and eggshell percentages, eggshell breaking strength (EBS), albumen height, and Haugh unit (HU). Data were analyzed using Proc GLM procedure of SAS appropriate for a factorial arrangement of treatments. Interactions between NPP and storage resulted in lower ($P < 0.05$) yolk percentage in fresh eggs from 100 and 110% NPP diets. Eggs from older hens had higher ($P < 0.05$) percent yolk but lower ($P < 0.05$) albumen and eggshell percentages, albumen height and HU. Fresh eggs had higher ($P < 0.05$) percent yolk and albumen, albumen height, and HU while the yolk color intensity increased ($P < 0.05$) with storage. Interactions between age and storage showed that yolk percentage was highest ($P < 0.05$) in stored eggs from 32 wk-old hens but lowest ($P < 0.05$) in fresh eggs from 24 wk-old hens. This interaction also shows that albumen height was highest ($P < 0.05$) in fresh eggs from 24 wk-old hens but lowest ($P < 0.05$) in stored eggs. The most intense ($P < 0.05$) yolk color was found in stored eggs from 24 wk-old hens with the lowest ($P < 0.05$) intensity found in fresh eggs irrespective of age at lay. Fresh eggs from 24 wk-old

hens had the highest ($P < 0.05$) HU with stored eggs from 32 wk-old hens having the lowest ($P < 0.05$) HU. The 2-way interactions between age and storage showed that stored eggs from 32 wk-old hens had higher ($P < 0.05$) EBS. At 24 wks, storage reduced ($P < 0.05$) Cu and Ca contents of the eggs. Egg Mn content decreased ($P < 0.05$) with 110% NPP in the diet. Interactions between storage and NPP level resulted in higher ($P < 0.05$) K in fresh eggs from hens on 90% NPP and stored eggs from hens on 110% NPP while P level in eggs was highest ($P < 0.05$) in fresh eggs from hens fed 110% NPP compared to stored eggs from hens on diet with 110% NPP. Egg Ca and Cu levels were higher ($P < 0.05$) in fresh eggs. This study showed a significant 2-way interaction between NPP and storage (percent yolk, K, and P) NPP and age (HU), age and storage (percent yolk, EBS, yolk color, albumen height, and HU).

Key Words: mineral content; egg quality; laying hens; egg storage; non-phytate phosphorous

154 The effect of different calcium and phosphorus recommendations and limestone solubility on early broiler performance and bone mineralization. Kyle Venter^{1*2}, Rosalina Angel³, Peter Plumstead^{4,2}, ¹Chemuniqué PTY Ltd, Lanseria, Gauteng, South Africa, ²Neuro Livestock Research, Brits, South Africa, ³University Of Maryland, College Park, Maryland, United States, ⁴Chemuniqué PTY LTD, Lanseria, South Africa, ⁵University of Pretoria, Pretoria, South Africa.

Limestone (LS) is the single largest contributor of Ca in broiler diets and differences in LS solubility have been shown to alter both Ca and P digestibility, thus potentially altering the requirement for these nutrients. As large differences exist in recommendations (REC) for Ca and digestible P (dP), the objective of this study was to evaluate two different REC. for Ca and dP and their interaction with LS of different solubility on early broiler performance. The study had 6 dietary treatments (TRT): 1-4 comprised a 2x2 factorial, with 2 Ca/dP REC University of Maryland (UMD) or Aviagen (2014) for pre-starter (0.42 dP, 0.96 Ca and 0.53dP, 0.94 Ca for the Aviagen and UMD respectively) diets (0 to 7-d) and starter (0.38 dP, 0.86 Ca and 0.40 dP, 0.89 Ca for the Aviagen and UMD respectively) diets (8 to 16-d). Diets were formulated using two LS sources with fast (93.1%) and slow (49.9%) solubility determined at 5 min based on the method of Kim et al. (2019). A phytase enzyme was included in these diets at 2000 FTU/kg feed with a formulated Ca and dP contribution of 0.188%. To separately assess the Ca and dP contribution assigned to the phytase, TRT 5 was formulated to the UMD Ca and dP REC with a slow soluble LS with no added phytase (Positive Control, PC). TRT 6 was a negative control (NC) formulated to contain 0.188% less Ca and dP vs. PC without phytase. TRT were assigned to 16 replicate pens in a randomized complete block design with 50 Ross 308 day-old mixed sex broiler chicks/pen. Body weight (BW), feed intake (FI), feed conversion ration (FCR) were determined

on 7-d and 16-d. On 10-d, two birds per pen were sampled for determination of defatted tibia ash as an indicator of bone mineralization. Data were analyzed in JMP 15.0 (SAS, Institute, Cary, NC) using a one-way ANOVA to compare the controls with the various diets. Effects of LS and Ca/dP REC and their interactions were analyzed using a 2-way ANOVA. Means were separated using Tukey HSD at $P \leq 0.05$. There were no interaction of Ca and dP REC with LS on BW or FCR on D7. Broilers fed UMD REC had higher 7-d BW (177g vs. 164g) and 16-d BW (617g vs. 596g) ($P < 0.05$) and lower 7-d FCR (0.96 vs. 0.87 g:g), with no difference in 16-d FCR (1.17 vs. 1.16). There was an effect of LS solubility on broiler BW in favor of slow soluble LS at 7d and 16d ($P < 0.05$). There were no differences ($P < 0.05$) between the PC and NC diets and the UMD diet with slow soluble LS for BW or FCR. The UMD REC improved ($P < 0.05$) 10-d tibia ash weight (469.63mg vs. 439.84mg) and slow soluble LS increased ($P < 0.05$) tibia ash weight (462.20mg vs. 447.28mg). These results suggest that early broiler performance and bone mineralization was affected by Ca/dP levels and the solubility of LS used.

Key Words: Calcium; Phosphorus; Limestone; Early nutrition

155 Effect of dietary calcium and phosphorus specifications and limestone solubility on performance, bone mineralization and cost of broiler production. Thabisa Soko^{1*}, Kyle Venter¹, Rosalina Angel², Mariana Ciacciariello⁴, Peter Plumstead¹, ¹Chemuniqué (PTY) Ltd, Lanseria, 1739, South Africa, ²University Of Maryland, College Park, Maryland, United States, ³Neuro Livestock Research, Kameeldrift, Brits, South Africa, ⁴University of KwaZulu Natal, Pietermaritzburg, KwaZulu Natal, South Africa.

Limestone (LS) solubility has been shown to alter Ca and P digestibility, in doing so potentially altering dietary requirements for these nutrients. The study objectives were to assess effects of different Ca and P recommendations (REC) on broilers performance, bone mineralisation and production costs when diets were formulated using LS with different solubility. A 2x3 factorial design included 2 LS solubilities (fast and slow), as per method of Kim et al. (2019). The Ca and P REC respectively from 2019 were pre-starter: University of Maryland (UMD) (0.94, 0.51); Aviagen (0.96, 0.48); Dutch nutrition group (NL) (0.84, 0.35). Starter UMD (0.89, 0.38); Aviagen (0.87, 0.44); NL (0.75, 0.29). Grower UMD (0.80, 0.28); Aviagen (0.87, 0.44); NL (0.75, 0.29). Finisher UMD (0.69, 0.28); Aviagen (0.79, 0.40); NL (0.75, 0.28). As hatched Cobb 500 chicks were placed in 72-floor pens, 50 broilers/pen and 12 replicates per treatment allocated using a randomized complete block design. BW and feed intake was determined at 10 and 32d and feed conversion ratio (FCR) calculated. Data were analysed in JMP 15.0 (SAS Inst. Inc., Cary, NC, 2016) by two-way ANOVA with block as a random effect and means separated with Tukey HSD at $P < 0.05$. Defatted

tibia ash was determined at 10 and 32d from 5 broilers/pen. Feed cost/kg broiler was calculated using commercial feed ingredient prices in South Africa, December 2021. BW at 10d was higher ($P<0.05$) on UMD (324g) REC vs. Aviagen (317g) and NL (317g) ($P<0.05$). FCR at 10d was improved ($P<0.05$) by the UMD REC vs. Aviagen and NL, with no effect of LS. At 32d, Aviagen (2038g) and UMD (2033g) were heavier than NL (1980g) ($P<0.05$). FCR at 32d for UMD was lower ($P<0.05$) than Aviagen and NL (1.33, 1.35, and 1.36, respectively). Slow soluble LS improved ($P<0.05$) 32d BW (2028 vs. 2006g) but had no impact on FCR. An interaction ($P<0.05$) was observed between LS and Ca and P REC on 10 and 32d tibia ash weight (wt). Fast soluble LS reduced ($P<0.05$) tibia ash wt in birds fed UMD REC at 10d but had no effect on tibia ash wt in birds fed NL or Aviagen

REC. At 32d, fast soluble LS reduced tibia ash in birds fed the NL REC, but not UMD or Aviagen REC. There was an interaction ($P<0.05$) for the total P consumed (g) per kg harvested where both NL treatments irrespective of LS and the UMD spec with a slow LS was improved ($P<0.001$). Both the Aviagen diets had a higher ($P<0.05$) P consumed (g) per kg harvested. There was a higher ($P<0.05$) Feed cost/kg broiler at 32d was improved by the UMD REC, followed by Aviagen and NL ($P<0.05$), with no effect of LS. These results suggest Ca and P REC and LS used in commercial broiler diets can impact live performance, bone mineralization and production costs.

Key Words: broiler; performance; sustainability; limestone solubility; bone-mineralisation

Metabolism and Nutrition, Feed Additives I

156 Impact of Saponin blend on broiler performance.

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Two trials were conducted to evaluate the anticoccidial activity of a saponin blend (Sarsap3), in battery raised broilers and second to evaluate efficacy in a used litter floor pen trial. For the first trial, 150 birds were placed in battery cages on day of hatch. There were 5 replications per treatment and 10 birds per cage. Treatment 1 and 2 were given unmedicated feeds throughout the trial. Treatment 3 was an unmedicated feed that included Sarsap3 (227 g/ton). On Day 12, birds in Treatment 2 and 3 were individually infected with 225,000 oocysts of mixed *Eimeria*. On Day 20, birds were sacrificed, and lesion scored. Performance and feed consumption was calculated. Data were subjected to ANOVA according to the General Linear Model (GLM). Tukey's multiple-comparison procedure was used to detect significant differences among dietary treatments. Treatment 3 birds had significantly lower ($p < 0.05$) average lesions (1.1) compared with Treatment 2 (1.7). Treatment 3 birds had similar FCR (2.153) as Treatment 1 (uninfected controls) (1.834) and significantly lower ($p < 0.05$) than Treatment 2 (2.733). Lesions were significantly less ($p < 0.05$) in Treatment 3 (1.9, 1.1, 0.3) for each intestine portion scored (duodenum, jejunum and ceca respectively) compared with Treatment 2 (2.7, 1.8, 0.7). Treatment 3 birds had similar weight gain (0.177) as Treatment 2 (0.135) and significantly less ($p < 0.05$) than Treatment 1 (0.235). (The second trial evaluated the impact of the saponin blend on broiler performance in a used litter pen trial. 552 day-old broilers were placed in two treatments. Treatment 1 was control with unmedicated feed and Treatment 2 had Sarsap3 (227 g/ton) in all feeds. 23 birds were placed in a pen and 12 replicates per treatment. Birds were raised until 42 days and performance was measured throughout the grow-out. Data were subjected to ANOVA according to the General Linear Model (GLM). Tukey's multiple-comparison procedure was used to detect significant differences among dietary treatments. No significant differences in performance were observed Day 0-14 ($p > 0.05$). No differences were observed in body weights for any period measured ($p > 0.05$). Treatment 2 (1.387) birds had significantly lower FCR Day 0-28 ($p < 0.05$) compared to Treatment 1 (1.398). Treatment 2 birds (1.566) had significantly lower FCR Day 0-42 ($p < 0.05$) compared to Treatment 1 (1.581). In conclusion, addition of this saponin blend improved FCR and reduced lesions from *Eimeria*.

Key Words: Broiler

157 Effect of plant-derived isoquinoline alkaloids on intestinal integrity and inflammation, stress response

and growth performance in broilers reared under heat stress conditions. Yongyuth Theapparat², Sunisa Khongthong², Damrongsak Faroongsarng², Natthrit Roekngam², Pipat Piewngam³, Waraporn Kraitavin⁴, Anja Pastor⁵, Tobias Steiner⁵, Marcos Rostagno^{1*}, ¹*Phytobiotics North America, Cary, North Carolina, United States*, ²*Prince of Songkla University, Songkla, Thailand*, ³*U.S. National Institutes of Health, Bethesda, Maryland, United States*, ⁴*Phytobiotics (Thailand) Co. Ltda, Bangkok, Thailand*, ⁵*Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany*.

Heat stress (HS) has been shown to negatively impact feed intake, body weight gain, and feed efficiency in broilers. Reduction of feed intake in birds exposed to HS is an adaptive mechanism regulated by the gut-brain axis, with the purpose of decreasing metabolic heat production. Plant-based isoquinoline alkaloids (IQs) have been used to support health, well-being and productive performance in poultry, especially under stress conditions. Therefore, a study was conducted to determine the effects of IQs from *Macleaya cordata* on intestinal integrity and inflammation, stress response and growth performance in broilers exposed to HS conditions. A total of 720 day-old Ross 308 broilers were assigned to 3 treatments (8 floor-pen replicates/treatment and 30 birds/replicate), including: 1) Negative Control (No IQs included), 2) IQs at 60 mg/kg of feed, and 3) IQs at 100 mg/kg of feed. Birds in all treatments were exposed to natural heat stress conditions (33-35°C and 70-75% humidity) in an open house research facility. Diets were based on corn and soybean meal, with feed and water provided *ad libitum* for the duration of the study (42d). The source of IQs was Sangrovit® Extra (Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany). Birds fed IQs had improved intestinal integrity, determined by significantly lower levels of FITC-dextran in plasma ($p < 0.05$), higher levels of occluding and mucin 2 ($p < 0.05$), at 35d. IQs also led to reduced intestinal inflammation at 35d, determined by significantly lower gene expression of NF- κ B, TNF- α and iNOS in the ileum ($p < 0.001$). Moreover, IQs significantly decreased levels of corticosterone and increased levels of serotonin in plasma ($p < 0.001$) at 35d. Birds fed IQs showed significantly decreased jejunal expression of neuropeptides ($p < 0.01$), such as cocaine- and amphetamine-regulated transcript (CART), cholecystokinin (CCK) and corticotropin-releasing hormone (CRH), resulting in higher feed intake ($p < 0.01$), compared to birds in the negative control treatment. Growth performance was improved by IQs, with birds showing higher body weight at 42d ($p < 0.001$), and a trend for better FCR at 42d ($p < 0.08$). Overall mortality was significantly lower in birds fed IQs ($p < 0.01$). In conclusion, this study demonstrates that supplementation of IQs from *Macleaya cordata* supports broilers under heat stress conditions, by protecting intestinal integrity, reducing

inflammation and improving growth performance, particularly through feed intake and body weight gain.

Key Words: Broilers; Inflammation; Heat stress; Feed additive; Isoquinoline alkaloids

158 Feeding flaxseed to chickens: does maternal fed flaxseed alter offspring brain docosahexaenoic acid and morphology? Rosemary Whittle^{1*}, David Ma¹, Elijah Kiarie¹, Alexandra Harlander¹, Tina Widowski¹, ¹*University of Guelph, Guelph, Ontario, Canada.*

Chickens can internally elongate omega-3 fatty acids (n-3 FA), converting alpha-linolenic acid (ALA) to the more functional docosahexaenoic acid (DHA), essential for brain development and maintenance. Priming eggs with n-3 FA supplies developing embryos with necessary DHA for brain development. We hypothesised that feeding flaxseed to chickens would increase brain DHA and alter brain size of their offspring. Ross 708 broiler breeders (BB) were raised in 8 pens and fed 4 maternal rearing - maternal laying (MR-ML) diet combinations of a 2.57% alpha-linolenic acid (ALA) rich flaxseed diet and control diet (control-control, control-flax, flax-control, flax-flax). At 30 and 33 weeks of age eggs were collected and incubated. Shaver white (W) and ISA brown (B) layer breeders (LB) were raised in 16 pens and fed flax (F) or control (C) maternal diet (MD); they received the same diet for the entire trial. At 30 and 36 weeks of age, eggs were collected and incubated. 40 chicks per BB MR/ML and 48 chicks per LB strain/MD were euthanised for brain dissections. Brain and body weight were recorded, and the ratio was calculated (B:BW). 6 chick brains per BB MR/ML and LB strain/MD were used for total fatty acid analysis to determine DHA content (N=48), reported as µg/0.1g brain tissue. Linear mixed effect models in R were used to analyse B:BW, and 2-way ANOVAs were used to analyse brain DHA. Broiler chicks from F ML had larger B:BW (9%) than C ML ($\chi^2=9.21$, $p=0.002$), and F MR tended to have larger B:BW (5%) than C MR broilers ($\chi^2=3.29$, $p=0.069$). There was no interaction between MR and ML for broiler B:BW. For layer B:BW there tended to be an interaction between strain and MD ($\chi^2=3.65$, $p=0.056$), however there were no significant pairwise comparisons ($p>0.1$) and no effect of strain or MD main effects ($p>0.1$). Mean DHA values for C-C, F-C, C-F, F-F broiler brains were: 427.6±38.0, 430.9±26.6, 458.8±50.8, 483.2±37.0, respectively, but effects of MR, ML and their interaction were not significant ($p>0.1$). Mean DHA values for B-C, B-F, W-C, W-F layer brains were 361.7±22.1, 454.9±33.7, 372.5±39.8, 397.5±16.8. F layer chicks (426.3±20.6) tended to have more brain DHA than C (366.6±21.8, $F=3.71$, $p=0.069$). There was no significant effect of strain or interaction between strain and MD for layer chick brain DHA ($p>0.1$). These results show that feeding BB flaxseed increased B:BW of their offspring, this was not seen for LB. Feeding flaxseed to LB increased brain DHA in their chicks, though lack of statistical difference in the BB study may be reflective of low sample size and not

dietary effects. In conclusion, we have shown that feeding flaxseed to breeders can increase the brain DHA and brain size of their offspring.

Key Words: Broiler; Chicken; Laying hen; Omega-3 fatty acids; Brain

159 Supplementation of a yeast fermentate on broiler performance and processing characteristics in commercial diets. Lindsey Wythe^{1*}, Dana Dittoe¹, Abe Schaeffer², Steven Ricke¹, ¹*University of Wisconsin-Madison, Verona, Wisconsin, United States*, ²*Harvest Fuel Inc, Walhalla, North Dakota, United States.*

Yeast fermentate (YF) products have been shown to have prebiotic-like properties by potentially altering bird growth and performance. The objective of this study was to evaluate the effects of supplementing a YF, ProBiotein® (PB), in commercial broiler diets on performance and processing characteristics. Dietary treatments consisted of a commercial least-cost formulated basal diet (Con), basal + 0.2% PB (0.2PB); basal + 0.5% PB (0.5PB); basal + 0.75% PB (0.75PB). On d0, a total of 400 Ross × Ross 308 males were randomly assigned to two battery brooders (block = 2) with 10 birds per pen and 20 pens per battery (n = 10, N = 400). Birds were offered feed and water *ad libitum*. On d14, birds were moved to floor pens following the same blocking design. Birds supplied the same treatment diets within a battery were combined into a floor pen (8 pens, 2 blocks, n = 45). Diet phases consisted of a starter (d0-14), grower (d14-28), and finisher (d28-42) with body weight (BW), feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR; corrected for mortality) collected weekly through d42. Processing occurred on d46 with live weight, carcass weight, absolute part weight, and part yield being recorded. Data were analyzed as a randomized complete block design using a linear regression model with treatment effects evaluated using a one-way ANOVA ($P \leq 0.05$). Pairwise differences were determined using Dunnett's test with an α -level of 0.05 (control = Con). There was a treatment effect on d 7 BW, where birds fed 0.2PB were larger than those fed Con ($P < 0.05$, 0.21 kg, 0.19 kg). From d 0-7, BWG was significantly greater in birds fed 0.5PB than those fed Con ($P < 0.05$, 0.16 kg, 0.17 kg). Birds fed 0.5PB had a significantly higher FI than those fed Con from d 0-14, ($P < 0.05$, 8.01 kg/pen, 7.58 kg/pen). However, birds fed 0.75PB had the numerically lowest FI from d 0-42, while birds fed Con had the highest ($P > 0.05$, 286.90 kg, 291.18 kg). There were no significant treatment effects in FCR throughout the study. Among processing characteristics, there was an effect of treatment on the yield of drums and tenders ($P < 0.05$). Birds fed diets with 0.2, 0.5, and 0.75PB had a lower drum yield than birds fed Con ($P < 0.05$, 11.68%, 11.82%, 11.83%, 12.46%, respectively). Similarly, the tender yield was lower in birds fed diets with 0.5PB than in birds fed Con ($P < 0.05$, 4.26%, 4.76%). In conclusion, the supplementation of PB in broiler diets improved early growth performance (d 0-7) and did

not negatively impact overall performance or processing characteristics. Further evaluations validating the benefits in poultry are warranted to determine the modality of the potential effects on early performance.

Key Words: performance; poultry; processing; yeast-fermentate

160 Effect of selenium on improving performance during high stocking densities in broilers. Jolien van Soest^{1*}, Brecht Bruneel¹, Theerawit Poeikhampa², ¹*Orffa Additives, Breda, Netherlands*, ²*Kasetsart University, Bangkok, Thailand*.

High stocking densities in broilers induce stress and impact performance and welfare. Selenium can be applied to reduce negative effects of stress. L-selenomethionine (organic selenium) can be stored in animal tissues and provides a continuous source of selenium, which supports the animal during high stress. The aim of this study was to investigate the effects of L-selenomethionine on improving performance whilst increasing stocking density in broilers. The trial included 1630 broiler chickens (Ross 308), over 6 treatments, 5 pens per treatment. Treatments included a control with sodium selenite (0.3 mg Se/kg feed) and treatment group with L-selenomethionine (Excential Selenium 4000, Orffa Additives BV) (0.3 mg Se/kg feed), for 3 stocking densities; standard stocking density (50 birds/pen or 29.84 kg/m²), +10% stocking density (55 birds/pen or 32.84 kg/m²) and +16% stocking density (58 birds/pen or 34.63 kg/m²). The birds were all fed the same corn and soybean meal-based pelleted diet, only difference being the type of selenium. Methionine was included to meet requirements. All diets satisfy the nutritional requirements described in strain's manual. The trial lasted 35 days (starter-finisher) and production parameters were recorded during the trial; body weight, feed conversion ratio (FCR), feed intake and mortality. After slaughter, meat quality and carcass composition were determined. Data was analyzed using ANOVA and Duncan's multiple range tests. After 10 days (starter period), body weight was slightly decreased when stocking density increased with 10% or 16%. Replacement of sodium selenite (318,3g) by L-selenomethionine (326,6g) increased body weight at all stocking densities applied ($p=0.0001$). For the overall period (starter-finisher), higher stocking densities had no negative influence on body weight and FCR. L-selenomethionine supplementation, in comparison with sodium selenite, showed improved body weight with 2377,59 kg and 2429,78 kg ($p=0.002$), body weight uniformity with 85,32 and 87,43 ($p=0.003$) and FCR with 1,350 and 1,327 ($p=0.04$), for sodium selenite and L-selenomethionine respectively, for all stocking densities applied. L-selenomethionine decreased shear force of breast meat from 4408,7 for sodium selenite to 3868,5 ($p=0.05$). Overall, the applied higher stocking densities did not negatively impact performance. Supplementation of L-selenomethionine instead of sodium selenite improved

performance consistently independent of the stocking density. Shear force (Warner-Bratzler method) was shown to be lower when L-selenomethionine was supplemented compared to sodium selenite. L-selenomethionine improves performance and flock uniformity in broilers and allows for a more economical production.

Key Words: broilers; stocking density; stress; performance; selenium

161 Effect of a proprietary plant extract-based product on growth performance and carcass yield of Cobb 500 or Ross 708 broilers. Shameer Rasheed^{1*}, Kristy Dorton¹, Gary Reznik¹, Mahmoud Masadeh¹, Mark LaVorgna¹, ¹*Devenish Nutrition, Fairmont, Minnesota, United States*.

Two studies were conducted to determine the effects of a proprietary plant extract-based product (EO, Devenish Nutrition, Fairmont, MN) on broiler growth performance and carcass yield. A total of 900 Ross 708 (Study 1) or 1,800 Cobb 500 (Study 2) 1-day old broiler chicks (as hatched) were randomly assigned to either 18 (Study 1; 50 birds per pen; 0.10 m²/bird) or 30 (Study 2; 60 birds per pen; 0.07 m²/bird) pens. There were 4 dietary phases in Study 1: starter (d 0 to 16), grower (d 17 to 31), finisher (d 32 to 45), and withdrawal (d 46 to 56) and 3 dietary phases in Study 2: starter (d 0 to 14), grower (d 15 to 26), and finisher (d 27 to 32). All diets were corn-soy-based pelleted diets. The treatment groups were: Control (basal diet, no test product) and EO at 0.10 g/kg (Study 1) or Control (basal diet, no test product) and EO at either 0.10 or 0.15 g/kg (Study 2). Bodyweight and feed consumption were measured at d 0, 16, 31, 45, and 56 (Study 1) or d 0, 14 and 32 (Study 2), and weight gain and feed conversion ratio (FCR) were determined. Data were subjected to a one-way ANOVA using the Mixed procedure of SAS 9.4 (2018) with pen as the experimental unit. Means were separated by Fisher's protected least significant difference. Differences were considered significant at $P \leq 0.05$. Bodyweight and weight gain were not significantly affected by treatment in either study except from d 32 to 45 in Study 1, where broilers fed EO at 0.10 g/kg had better ($P=0.01$) weight gain compared to broilers in the Control group. In Study 1, broilers supplemented with 0.10 g/kg tended to have better FCR from d 17 to 31 ($P=0.06$; 1.425 vs. 1.448), d 32 to 45 ($P=0.009$; 1.775 vs. 1.831) and d 0 to 45 ($P=0.05$; 1.518 vs. 1.544) compared to the Control group. In Study 2, broilers supplemented with 0.15 g/kg of EO had better ($P=0.03$; 1.476 vs. 1.495) FCR from d 0 to 32 compared to broilers in the control group. Broilers supplemented with 0.10 g/kg had numerically better FCR than control ($P=0.13$; 1.479 vs. 1.495). Additionally, broilers supplemented with 0.10 g/kg EO reduced the number of woody breasts (0% vs. 42.86%) compared to the control group in Study 1. Overall, the results suggest that supplementation of EO helped to improve FCR (up to 5.6 points) and reduced the incidence of woody breast in large (Ross 708) broilers.

Key Words: broiler; growth performance; woody breast; plant extract

162 Evaluation of an encapsulated calcium butyrate on serum antioxidative characteristics, gut barrier function and inflammatory responses in broiler chickens under a necrotic enteritis challenge. Jinquan Wang^{1*}, Jundi Liu², Janghan Choi¹, Hanyi Shi¹, Amit Singh¹, Hanseo Ko¹, Luke Bernard², Brett Lumpkins³, Greg Mathis³, Woo Kim¹, ¹*University of Georgia, Athens, Georgia, United States*, ²*Eastman Chemical Company, Kingsport, Tennessee, United States*, ³*Southern Poultry Feed & Research Inc, Athens, Georgia, United States*.

To further understand the mode of action of fat encapsulated calcium butyrate (Ca-B) in broiler chickens under a necrotic enteritis challenge, a total of 2,000 Cobb-Cobb off-sex male broilers were placed on the day-of-hatch with 50 chicks per floor pen and 10 replicates pens per treatment. The study consisted of 4 treatments: non-challenged control, challenged control, challenged control + Ca-B low-dose (200/150/150 mg/kg for the starter/grower/finisher phase, respectively) and challenged control + Ca-B high-dose (350/250/250 mg/kg). All birds were sprayed with 1x commercial coccidiosis vaccine on day 0. Birds from the challenged treatments were inoculated in feed with ~5,000 oocysts of *Eimeria maxima* on day 14, followed by 10⁸ cfu/mL *Clostridium perfringens* on day 19, 20 and 21. On day 21 and 28, 1 bird per pen was randomly selected to collect blood, jejunum, and cecal tonsil for further sample analyses. Data were analyzed using one-way ANOVA model for a completely randomized design using the GLM procedure of SAS 9.4. Significant level was set at $P < 0.05$. Results showed that birds from the challenged control had significantly higher serum malondialdehyde (MDA) and lower total antioxidant capacity (Trolox equivalent, TAC) than the non-challenged control on day 21 ($P < 0.05$). Supplementation of Ca-B at both low- and high-dose significantly reduced serum MDA and increased TAC to the same level as the non-challenged control ($P < 0.05$). Additionally, birds fed with the high-dose Ca-B diet showed a significantly higher superoxidative dismutase (SOD) activity compared to the challenged control ($P < 0.05$). Mucin 2 gene expression was downregulated in the challenged broilers compared to the non-challenged group. No significant difference was found among non-challenged control, low-dose Ca-B, and high-dose Ca-B on mucin 2 gene expression on day 21. Meanwhile, Ca-B downregulated the gene expression of nuclear factor-kb in the ceca tonsils on both day 21 and 28 compared to the non-challenged control ($P < 0.05$). In conclusion, Ca-B supplementation at both low- and high-dose was able to benefit birds from the necrotic enteritis by improving serum antioxidant capacity, affecting mucin production, and regulating inflammatory cytokines gene expressions.

Key Words: broiler; necrotic enteritis; calcium butyrate

163 Effect of Stimbiotic as a gut microbiome modulating additive on performance and productivity in broilers. Alexandre de Brito^{1*}, Fabio Valle¹, Eduardo Afonso Frandi Butolo³, Xavière Rousseau¹, Gilson Gomes², ¹*AB Vista, Marlborough, Wiltshire, United Kingdom*, ²*AB Vista, Plantation, Florida, United States*, ³*JEB Agropecuária LTDA, Mogi Mirim, Brazil*.

Antimicrobial growth promoters (AGP) have long been used to improve performance in poultry industry. However, due to concerns of antimicrobial resistance, the use of AGP has been reduced or banned in many countries. In this way, Stimbiotic (STB) additive has been introduced recently and defined as non-digestible but fermentable additives that stimulate fiber fermentability but at a dose that is too low to directly contribute in a meaningful manner to volatile fatty acid production. Therefore, unlike prebiotics that are quantitatively fermented by the microbiome, STB simply stimulates fibrolytic microbiome establishment and therefore the fermentation of dietary fiber. The objective of this trial was to evaluate the STB compared with other additives present in Brazil. A total of 1500 Cobb 500 broilers were housed in a completely randomized design in the experimental unit of JEB Agrop. in 30 pens of 50 birds each (500 birds/treat) from 0 to 42 days old, challenged by coccidiosis vaccine. The dietary treats were 1) Diet with MOS (Manano-oligosaccharides) + Butyrate + Muramidase + Carbohydrase ; 2) Diet with only MOS + STB; 3) Diet with only Butyrate + STB. All diets were based on corn and soybean meal and animals, and the nutritional requirements was based in Brazil Table (2017). Body weight gain (BWG), feed conversion corrected for mortality (mFCR), liveability, European production efficiency factor (EPEF) and feed production cost were evaluated. Animal performance results were subjected to one-way analysis of variance using JMP Pro 15 (SAS). The results of performance and average feed cost was: BW in day 0, g/bird: 45.65, 45.71 and 45.54, for Treat 1, 2 and 3 respec. ($P=0.92$); BWG in day 42, g/bird: 2.895^a, 2807^b and 2836^{ab}, for Treat 1, 2 and 3 respec. ($P=0.07$); mFCR in day 42, g/g: 1.61^a, 1.68^b and 1.63^a, for Treat 1, 2 and 3 respec. ($P=0.07$); Liveability in day 42, %: 95.2, 93.8 and 95.8, for Treat 1, 2 and 3 respec. ($P>0.05$); EPEF in day 42: 408^a, 375^b and 396^{ab}, for Treat 1, 2 and 3 respec. ($P=0.08$); and Average feed cost, US\$/ton: 220, 218 and 217 for Treat 1, 2 and 3 respec. The results indicate a trend towards reduced performance when replacing the four additives used in Treat. 1 with STB + MOS (Treat 2). However, this trend does not occur in the association of STB + Butyrate (Treat 3). These results may be indicative of the mode of action of STB, due to its interaction with dietary fiber produces XOS fractions, it generates an effect similar to that of MOS, which may be non-synergistic; however, this effect may have synergism with other additives commonly used in diets; generating performance maintenance with a cost of up to US \$ 2.00/ton lower!

Key Words: Performance; Broiler; Stimbiotic; average feed cost; gut microbiome modulating

164 Effect of a chicken-derived microbial feed supplement on intestinal microbiome and performance of broilers. Brian Dirks^{1*}, Joshua Lefler¹, Andrew Izzo¹, James Gaffney¹, Kayla Calapa¹, Jolie LoButto¹, Allison Wells¹, Mallory Embree¹, ¹*Native Microbials, Norristown, Pennsylvania, United States.*

Two successive pen trials were conducted to determine the effectiveness of a *Bacillus amyloliquefaciens* strain (Avius Protect™, Native Microbials, Inc.) on broiler performance and intestinal microbiome population when administered in feed to straight run broilers. This chicken-derived strain is a colonizing, native species of the intestines that was selected from high performing broilers. The first trial consisted of Cobb 500 straight run broilers in 5' x 5' pens consisting of 31 broilers per pen and 16 replicates per treatment. Treatments consisted of a normal energy feed, a limited energy feed, and a limited energy feed with the addition of the strain. An energy limited diet was employed to facilitate the ability to show differences in performance absent disease challenge. The trial was conducted from hatch to day 28. The second trial was performed at the same location with the same treatments but concluded on day 49 and consisted of 20 replicates of a final count of 28 birds per pen. Body weight gain (BWG), feed intake (FI), feed conversion (FCR) and mortality adjusted FCR (mFCR) were collected. For both trials, small intestinal contents were collected for microbiome analysis on days 14 and 28. Statistical analysis was performed using a combination of one-way ANOVA, followed by Fischer's least significant difference (LSD) with bonferroni correction for p-value adjustments. Microbiome analysis was conducted using Usearch (v11.0) for sequence processing, OTU generation and subsequent analysis. Differential abundance analysis was performed using DeSeq2 (v3.14). The initial 28-day trial resulted in a statistically significant ($p < 0.01$) mFCR improvement of 0.05 compared to the limited energy control. The second 49-day trial resulted in a statistically significant ($p = 0.02$) mFCR improvement of 0.03 compared to the limited energy control. Microbiome analysis revealed a reduction in microbiome alpha diversity in the small intestine of birds fed the *B. amyloliquefaciens* compared to the low energy control. Additionally, several OTUs – belonging to families such as *Lactobacillaceae*, *Bacillaceae*, *Enterobacteriaceae* – statistically increased in treated birds when compared to control birds. These results indicate that this new *B. amyloliquefaciens* isolate was able to have a positive impact on broiler performance when added to the diet.

Key Words: Performance; Probiotic; Broiler; Microbiome; Bacillus

165 Effect of soluble and insoluble fiber in interaction with the probiotic *Bacillus amyloliquefaciens* CECT

5940 on the microbiota dynamic of broilers from day 1 to 35. Nadia Yacoubi^{1*}, Martina Klünemann¹, Carine Adams², Catarina Stefanello², ¹Evonik Operations GmbH, Hanau- Wolfgang, Germany, ²Federal University of Santa Maria, Santa Maria, Brazil.

During the recent years the increasing prices of feed raw materials resulted in the use of new ingredients in animal production. These new ingredients often have a high amount of fiber that can result in lower performance and intestinal health issues. Feed additives as probiotics can help overcome this negative impact, improving the economic success of diets formulated with alternative ingredients. In this study, the objective was to investigate the effect of fiber in interaction with probiotic on the cecal microbiota dynamic of broiler from day 1 to 35. A total of 672 one-day-old male Cobb 500 slow feathering broilers were used. Dietary treatments were distributed in a 2 x 2 factorial arrangement, with 6 replicates and 28 broilers each experimental unit. Two diets with high (0,3) and low (0,16) ratio of soluble to insoluble fiber, not supplemented or supplemented with the probiotic *Bacillus amyloliquefaciens* CECT 5940. Cecal microbiota composition (using 16s sequencing) were measured at day 7, 14, 21 and 35. Microbiota data was analyzed using R3.6.3 with base and vegan libraries. The species richness was positively correlated with age and with probiotic supplementation. The differential abundance analysis showed that with time we observed a decrease in the relative abundance of the *Clostridiales* order mainly the *Lachnospiraceae* family, and an enrichment of the *Bacteroidales* order mainly the *Rikenellaceae* family. We observed a significant increase of *Faecalibacterium* until day 21, then it decreased again in day 35. *Alistipes* genus was increased with time. On day 7, the abundance was between 0.65 and 1.4% when broilers were fed diets not supplemented or supplemented with the probiotic, respectively, reaching 20.05 and 27.75% at day 35, respectively. When comparing the microbiota abundance on day 7, it was observed that groups supplemented with probiotic reached a comparable composition to the group without probiotic in day 14, which may suggest that the probiotic accelerated the maturation of the microbiota during the early stages. Main difference between the fiber sources was a statistically decreased abundance of *Lachnospiraceae* in groups fed a higher ratio soluble/insoluble fiber at day 7 and 14. In conclusion, in this study it has been shown that the maturation of the cecal microbiota in broilers is mainly driven by time and then by the feed ingredients and feed additives, particularly the probiotic.

Key Words: microbiota; probiotic; Broiler; fiber

166 Evaluation of a *Bacillus*-based direct-fed microbial on broiler performance and excreta properties when fed in diets with or without DDGS. Hector Leyva-Jimenez^{1*}, Yemi Burden¹, Katherine

McCormick¹, Adrienne Woodward¹, Mike Blair¹, ¹*United Animal Health, Inc., Sheridan, Indiana, United States.*

The implementation of feed amendment strategies to reduce noxious odors from animal operations has intensified over the last few years due to the increasing concerns over environmental pollution. Direct-fed microbials (DFM) can reduce the production and emission of noxious gases such as ammonia (NH₃) when offered in poultry diets by modifying the properties of the excreta and regulating its decomposition. The objective of the present study was to evaluate the *Bacillus*-based DFM Amnil® (United Animal Health Inc., Sheridan, IN, US), on broiler body weight gain (BWG), feed intake (FI), mortality corrected feed conversion ratio (FCR), livability (LV), excreta pH, and excreta nitrogen (N) analyzed as total N (TN) and ammonium N (NH₄-N). A total of 216 newly hatched by-product male broiler chickens were individually weighed and allotted to stainless steel batteries to create 36 groups of 6 chickens. Dietary treatments were randomly assigned to cages in a 2 x 2 factorial arrangement using a complete randomized design. There were 9 cage replicates per treatment. The birds were raised inside an environmentally controlled room where feed and water were available ad libitum. Treatments consisted of 2 diet types (Corn-SBM; CS or Corn-SBM+DDGS; CSD) and 2 inclusion rates of DFM (0 or 500 ppm; target to yield 7.4x10⁴ CFU/g of feed). A 3-phase feeding program was employed (Starter 0-14 d, Grower 14-28 d, and Finisher 28-35 d). In the CSD diet, DDGS inclusion was fixed at 5% in the starter and grower diets while 10% was used in the finisher diet. Cumulative performance and excreta properties were evaluated at 35 d. Data were analyzed in SAS using two-way ANOVA with diet type and DFM inclusion as fixed factors (Livability analyzed by Kruskal-Wallis test). After 35 d, independently of diet type, the BWG of broilers was higher (*P*<0.05) by 5%, and the FCR was improved (*P*=0.099) on average by 5 pts when the DFM was offered in the diet. Better LV of broilers fed the DFM (*P*<0.05) was identified. The NH₄-N was significantly increased (*P*<0.05) in excreta from broilers fed CSD+DFM. A numerical increase in NH₄-N was observed in the excreta from the CS+DFM group when compared to CS alone. A reduction (*P*<0.05) in pH was observed when DFM was offered in CS diets but no effects were observed in CSD diets. Excreta TN was numerically reduced in both diet types when the DFM was fed. In conclusion, DFM inclusion in the diet improved the performance of broilers fed CS or CSD diets. Moreover, regardless of diet type, lower TN in excreta from broilers fed DFM suggests lower N available for ammonia (NH₃) production, while higher NH₄-N in the excreta of DFM fed broilers suggest higher N retained and less N volatilized as NH₃.

Key Words: broiler; direct-fed microbial; performance; *Bacillus*; excreta

167 The effect of a *Bacillus*-based direct-fed microbial on the pH and total aerobic microbial counts in broiler excreta— a case study. Karely Cantu^{1*}, Hector Leyva-Jimenez¹, Beth Galbraith², Michael Martin¹, Adrienne Woodward¹, Mike Blair¹, ¹*United Animal Health, Inc., Sheridan, Indiana, United States*, ²*Microbial Discovery Group, Franklin, Wisconsin, United States.*

Direct-fed microbials (DFM) designed to thrive under a wide variety of conditions are optimally positioned to extend their beneficial effects to animals beyond the inner intestinal environment by regulating the microbial decomposition of excreta. The objective of the present study was to evaluate chicken excreta properties and total aerobic microbial counts in the presence or absence of a DFM. Two excreta samples were collected from 108 by-product male broilers at the grower phase allocated in battery cages (6 birds per cage and 9 cages per treatment). The first sample was from birds with no DFM in the diet (CON); the second sample was from birds consuming 250 ppm DFM in the diet (NF; Novela®, United Animal Health, Sheridan, IN, US). The CON excreta was then subdivided into 3 subsamples; 1) remained as CON, 2) excreta directly inoculated with 0.4 g DFM (NI) targeting the inclusion rate as offered through the feed, and 3) excreta inoculated with 8.0 g DFM (NI20) targeting 20x the inclusion level of NI. The DFM inoculation rates for NI and NI20 were calculated under the assumption that feed digestibility in the intestine of chickens would be around 90% thus, ~1.5 kg feed would yield 450 g of excreta with dry matter content of 34.25%. Four replicate (450 g) excreta samples per treatment were weighed, placed in sealable 1.4 L plastic containers, and inoculated with the DFM. To maintain moisture, 200 mL of diH₂O were added to each sample and homogenized. All samples were incubated in a temperature-controlled room at 30°C for 48 h. The pH was evaluated before incubation (time 0), and after 12, 24, 36, and 48 h of incubation. For each treatment, an excreta composite was created before and after 48 h of incubation to evaluate total aerobic microbial counts. Five excreta samples were aseptically collected at random per treatment composite and plated onto 3M aerobic plate count (APC) Petrifilms. The pH data were subjected to ANOVA using the proc mixed procedure of SAS and means were compared using Tukey's test (*P*<0.05). Results indicated that on average, the NF treatment had a higher pH (5.50; *P*<0.05) throughout the incubation period compared to the other treatments (CON 5.23, NI 5.20, and NI20 5.33). In addition, the APC results showed that the NF treatment had higher microbial counts (9.71 log₁₀ cfu/g) after 48 h of excreta incubation compared to all other treatments (CON 8.68, NI 8.92, and NI20 9.02 log₁₀ cfu/g). The APC results for NI and NI20 were higher than the CON treatment after the 48 h incubation period. The data collected from this study suggested that the *Bacillus* present in the evaluated DFM may persist in the excreta and maintain an active aerobic microbial activity.

Key Words: broiler; pH; Direct-fed microbial; excreta; aerobic bacterial count

168 Effects of a phytogenic blend on broiler growth performance under a *Clostridium perfringens* challenge. Meghan Schwartz^{1*}, Stacie Crowder¹, ¹PMI, Louisburg, North Carolina, United States.

A study was conducted to evaluate the effects of a phytogenic feed additive on broiler performance under a *Clostridium perfringens* challenge. A total of 972 day-old Cobb 500 males were assigned to 3 treatments with 12 replicate pens per treatment (27 birds/pen) in a complete randomized block design. Block was based on pen location within research facility. Treatments were 1) unchallenged control (UC), 2) challenged control (CC), and 3) CC with SupplantTM D (PHYTO) at 150 mg/kg (PMI, Arden Hills, MN). At day 17, birds in CC and PHYTO treatments were indirectly challenged with *Clostridium perfringens* at 4.88×10^{10} CFU/kg of feed. At 21 days, 5 birds per pen were euthanized and evaluated for intestinal lesions and necrotic enteritis. Body weight gain (BWG), feed intake (FI), and feed conversion ratio adjusted for mortality (mFCR) were evaluated at 17, 28, and 35 days of age. Statistical analysis was performed using Proc Mixed procedure in SAS and significant means were compared by Least Significant Difference ($P < 0.05$). Throughout the study, no significant effect on FI and mortality was observed. At 17 days age, PHYTO treatment improved mFCR compared to UC and CC groups (1.261 vs 1.275 vs 1.285, $P = 0.0188$). The *Clostridium perfringens* challenge worsened lesion scores at 21 days of age in the CC and PHYTO treatments compared to the UC treatment (1.42 vs 1.35 vs 0.00, $P < 0.001$). Furthermore, no significant effects on BWG and mFCR were observed at 28 days. At 35 days of age, PHYTO treatment improved mFCR compared to UC and CC groups (1.542 vs 1.562 vs 1.566, $P = 0.0309$). The phytogenic blend numerically increased BWG at 17 and 35 days of age when compared to UC and CC treatments. Based on these results, including this phytogenic blend in broiler diets during a *Clostridium perfringens* challenge supports improved mFCR.

Key Words: broilers; necrotic enteritis; feed additive; feed conversion; phytogenic

169 Effect of a benzoic acid on growth performance of broilers challenged with *Clostridium perfringens*. Liris Kindlein^{1*}, Andre Favero², Sergio Vieira¹, Ross

Wolfenden³, Patrícia Aristimunha⁴, Flavio Longo⁴, ¹UFRGS, Porto Alegre, RS, Brazil, ²Santa Livia Farm, Farroupilha, RS, Brazil, ³Eastman USA, Kingsport, Tennessee, United States, ⁴Eastman Brazil, São Paulo, SP, Brazil.

An experiment was conducted to evaluate the dose response of benzoic acid (BA) in a necrotic enteritis (NE) model. A total of 1,320 one-day-old male Cobb x Cobb 500 slow feathering broiler chicks were allocated in 60 floor pens (1.5 x 1.5 m), placed on six times reused pine shavings as bedding. Treatments were distributed in a completely randomized design with 6 treatments with 10 replicates of 22 chickens each. The experimental treatments were NC: negative control (AGP free), PC: Positive control (avilamycin – 10ppm); NC + BA 0.25kg/MT; NC + BA 0.50kg/MT; NC + BA 1kg/MT and NC + BA 2kg/MT. All broilers were vaccinated for coccidiosis at placement using a 2x dose. The broilers were also orally inoculated with a pathogenic strain of *Clostridium perfringens* type A on days 11, 12, and 13 after placement using the concentration of 10^8 CFU/mL/bird. Data were analyzed using one-way ANOVA and means were separated using Tukey's test ($p < 0.05$) using SAS 9.0 (2009). Additionally, a contrast analysis (NC or PC vs. BA) was conducted. No significant differences ($p > 0.05$) were observed on cumulative body weight gain, on cumulative feed intake on all period (1-41 days). A significantly decreased feed conversion rate was observed in the BA 2kg/MT treatment compared to the negative control (1.282 vs. 1.335) in the period from 8 to 14 days of age. All treatments with BA showed better cumulative feed conversion rate than negative control ($p < 0.05$) in the period from 1 to 28 and from 1 to 41 days. Considering overall measured performance parameters (weight gain, feed intake, feed conversion rate and mortality) it was observed by contrast analysis that birds fed BA presented the same results on IEP (productivity index) and on nitrogen ileal digestibility (%) compared to the PC and significantly better results compared to the NC treatments ($p < 0.05$), proving the benefit of the benzoic acid on supporting the birds under this challenge protocol without inclusion of AGP. In conclusion, according to these results, the benzoic acid fed to the broilers in this trial performed similarly to the PC treatment under the challenge protocol using *Eimeria* vaccine and *Clostridium perfringens* inoculation and should be considered an effective alternative to AGP under these conditions.

Key Words: *Clostridium perfringens*; growth performance; benzoic acid; AGP-Free; nitrogen ileal digestibility

Management and Production

170 Effect Of different levels of light intensity on behaviour and blood profile of broilers. Asad Khan^{1*}, Ghulam Abbas Hashmi¹, ¹Riphah College of Veterinary Sciences, Lahore, Pakistan.

Commercial birds at farms in Pakistan are often reared at higher light intensities than the actual need thus negatively affect the welfare of birds as well as profit margin of farmers in terms of extravagant use of energy. So we designed this project to explore most responsive light intensity for chicken. One hundred fifty broiler chicks were randomly divided into 15 experimental units comprising 10 chicks each, to be designated as replicates, three replicates were randomly assigned to each of the five experimental treatment groups. All birds were provided 20 lux light during first week after that treatment distribution was as follows. Light treatment T1 i.e. (5 lux from 2-6week) was given to group A, T2 i.e. (10 lux from 2-6week) was given to group B, T3 i.e. (20 lux from 2-6week) was given to group C, T4 i.e. (30 lux from 2-6week) was given to group D, T5 i.e. (40 lux from 2-6week) was given to group E. Chicks were vaccinated according to recommended schedule. All birds were provided homogeneous environmental conditions except light intensity. The data collected for behavioral profile, blood profile, rectal temperature and respiration rate were statistically analyzed using Completely Randomized Design. Results revealed non-significant ($P > 0.05$) effect of light intensity on: eating behavior at Week (W) 2, W4, W5, W6., drinking behavior at W2, W5, W6., standing behavior at W2, W5., Preening behavior at W2, W3, W5, W6 and aggressive behavior at W3, W4, W5. whereas; Eating behavior at W3, drinking behavior at W3, W4, standing behavior at W3, W4, W6, Preening behavior at W4 and aggressive behavior at W2, W6 were affected ($P < 0.05$) by light intensity. Light intensity affected cholesterol and T4 hormone level ($P < 0.05$) whilst it did not affect cortisol, urea, creatinine, T3 level, respiratory rate and rectal temperature ($P > 0.05$). The present research will cause a significant impact on poultry industry of Pakistan. The research has provided an important clue about the obligatory light intensity for best production performance in the ecological conditions of Pakistan. It is recommended that light intensity at broiler shed should not exceed than 5 lux.

Key Words: Broiler chicken; Light Intensity; Blood Profile

171 Lighting requirements of broilers raised without antibiotics. Tory Shynkaruk^{1*}, Karen Schwean-Lardner¹, ¹University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Raised without antibiotics (RWA) broiler production has become an increasing global trend. Moving from conventional rearing to RWA can have detrimental effects on bird productivity. Adaptation of management practices

can aid in minimizing the negative impacts associated with RWA flocks. While it is known that providing a dark period improves productivity (growth rate, feed efficiency, and reduced flock mortality) in conventionally reared birds, it is unclear what benefits will be realized in RWA flocks. Therefore, the objective of this research was to assess the impact of photoperiod on the productivity of RWA broilers. Two identical blocked trials were conducted, each using 4,032 Ross 308 broilers ($N=8,064$) reared to 36 d. Birds were housed in 8 identical environmentally controlled rooms with 12 pens per room at a stocking density of 21 kg/m². From 6 to 36 d, lighting treatments were 14L:10D, 17L:7D, 20L:4D, and 23L:1D. Each treatment was replicated in two rooms per trial resulting in 4 room replications overall. Body weight (BW), feed intake and feed efficiency were calculated at 7, 21, and 36 d. Flock uniformity was assessed at 34 d ($n=336$ birds per treatment per trial). Mortality was monitored daily and birds were necropsied by an independent laboratory to determine cause of mortality. Data were analyzed using regression analyses (Proc Reg and Rsreg of SAS 9.4). Differences were considered significant when $P \leq 0.05$. A quadratic relationship between BW and treatment existed at 7 ($P < 0.01$), 21 ($P < 0.01$), and 36 d ($P < 0.01$) with the highest final BW at 36 d under 20L. Feed consumption responded quadratically at all periods with birds on 20L consuming the most feed. Feed efficiency with mortality correction responded in a linear fashion from 21-36 d and 0-36 d, with longer dark periods resulting in improved feed conversion. There was no effect of photoperiod on mortality. A quadratic response was observed for the percentage of birds within 5% of the mean BW for uniformity ($P=0.05$) with the highest percentage under 23L. A linear increase with increasing photoperiod was observed for the percentage of birds within 15% of the mean ($P=0.04$) with higher uniformity under 23L. In conclusion, the results indicate that including a minimum 4 h of continuous darkness in the lighting programs of RWA broilers improves performance.

Key Words: management; production; no antibiotics ever (NAE); antibiotic free (ABF); daylength

172 Effects of broiler stocking density on growth performance and muscle myopathies in Ross 708 female broilers. Clay Maynard^{1*}, Casey Owens¹, ¹University of Arkansas, Fayetteville, Arkansas, United States.

Stocking density of broilers in commercial practice has recently gained pertinent observation. Integrators are attempting to find ways to maximize profit, while reducing fixed processing costs and losses within the plant. Specifically, meat quality has become a focal trait that processors are now investigating. Being able to reduce economic loss through a reduction in severe myopathies such as woody breast (WB) or white striping (WS) would then produce a higher return. Therefore, the purpose of the

current experiment was to assess three commercial stocking densities on the occurrence and severity of breast muscle myopathies. 432 female Ross 708 female broilers were reared for 52 days amongst eighteen pens to give six replicates per treatment. Treatments consisted of 0.10 sq m/bird, 0.09 sq m/bird, and 0.08 sq m/bird to give a low, standard, and high level of density, respectively. Dead or cull birds were replaced throughout the trial to maintain density but were excluded from processing. For data analysis, pen served as the experimental unit and a mixed model was used to assess the effects of treatment on yields, WS and WB occurrence with significance deemed at $P \leq 0.05$. In accordance, breast weight was higher ($P < 0.05$) in the low treatment when compared to both the standard and high treatments. For WS, a difference ($P < 0.05$) was observed as the standard treatment expressed the highest mean score, the high density the lowest, and the low density was intermediate. For WB, a difference ($P = 0.054$) was observed between the high treatment and both the low and standard treatments, as no differences were expressed between the two. A difference ($P < 0.05$) was observed in WS occurrence for a score of zero, as the high density expressed the highest occurrence of zero scores, standard being the lowest, and the low density intermediate. No differences ($P > 0.05$) in WS were observed for scores of 1 or 2. No differences in mean score ($P > 0.05$) were observed in WB for any of the three densities. Overall, the effects of stocking density had a minor influence on the final presence of breast myopathies. Further investigation on the effects of increased stocking density on meat quality should be assessed to aid in integrator/processor return.

Key Words: meat quality; stocking density; white striping; woody breast; high yielding

173 The potential of traps and lures to monitor for bed bug infestation on commercial egg farms. Paul Patterson^{1*}, Karen Poh¹, Nuket Acar¹, Gregory Martin², Max Pasquinelli¹, Madison Berger¹, Mia Esoldo¹, Kylie Green¹, Nina Jenkins¹, ¹*Penn State University, University Park, Pennsylvania, United States*, ²*Penn State Extension, Lancaster, Pennsylvania, United States*.

Bed bug (BB) infestations of commercial poultry are challenging for the birds and care-takers because of the health, irritation and cost to rid poultry facilities and homes of this pest. In poultry facilities, attraction effectiveness is hampered by thousands of poultry hosts, high CO₂, dust, dander and unlimited harborage. Our objective was to evaluate commercial traps and lures for potential to monitor BB numbers and locations on commercial egg farms. Five traps were evaluated for their potential to capture BB on 4 farms with packing rooms and some history of infestation. Three farms had 4 cage-free houses for fertile eggs and 1 farm had 3 caged houses for table eggs. Treatment traps (Volcano+SenSci lure [VS], Volcano+Socks lure [Socks], Nattaro Scout+lure, Delta-5, Cardboard [CB]) were launched in each house with 3-6 reps/treatment depending

on house type (T-0). At 2 wk intervals the trap contents were collected and replenished (T-1 & T-2). At T-3, traps and their contents were collected and removed from the houses. Following each collection, BB were enumerated as 1st-3rd stage nymphs, 4th-5th stage nymphs, adult females and males. The data were analyzed by one or two factor ANOVA using PROC GLM procedures of SAS Institute Inc. after utilizing arcsine sqrt transformation of the percentage data. Significance was set at ($P < 0.05$). In one table egg house at T-1, BB were greater in the center and left wall cage rows than the right, respectively for percent 4th-5th stage nymphs ($P = 0.0527$) and adult females ($P = 0.0688$). However, there was no differences between the treatment traps during any time period ($P > 0.05$). On the 1st cage-free farm there were only 34 BB trapped at T-1 with none found in the CB, Delta-5 or Nattaro traps. However, the trend was for the VS to trap more than Socks and significantly so for adult males (8.82 vs. 0/trap, respectively; $P = 0.032$). On the 2nd cage-free farm at T-1, adult females in the pit were trapped at a greater percent using the Socks compared to the Delta-5 ($P = 0.054$) but, there were no further differences at T-2 or T-3. At the 3rd cage-free farm, total BB numbers increased over time from 1484 to 3028 and 5416 at T-1, T-2 and T-3, respectively. While at T-1 there were no trap differences; at T-2 Socks had more 1st-3rd and 4th-5th stage nymphs, females, males and total BB per trap than the CB or Delta-5 ($P < 0.05$). At T-3 the VS trapped more 1st-3rd stage nymphs ($P = 0.054$) and total BB ($P = 0.058$) per trap than the other treatments. In summary, houses with BB saw increasing numbers over time and the VS and Socks trapped more per trap than the Nattaro, Delta or CB. However, the traps did not pick up significant BB in packing rooms or break rooms on any farm.

Key Words: integrated pest management; Commercial poultry; bed bugs; traps; lures

174 Determining the microbiota composition of poultry litter during an extended downtime between flocks. Dana Dittoe^{1*}, Chase Miller², Kevin Kessler³, Steven Riche¹, ¹*University of Wisconsin-Madison, Madison, Wisconsin, United States*, ²*Elanco US, Greenfield, Indiana, United States*, ³*Tyson Foods, Springdale, Arkansas, United States*.

Managing poultry litter between flocks remains a challenge for the commercial poultry industry as downtime between flocks varies significantly. Therefore, the objective was to determine the impact of brooding (brood vs non-brood), location (feedline vs middle), and an extended downtime (28d) on the litter diversity and microbiota composition at a commercial broiler house practicing half-house brooding. Immediately following the removal of the previous market age flock, litter was collected every 7 d until the new flock was placed (0, 7, 14, 21, 28d) and 3 and 7d after chick placement (31 and 35d). Samples were repeatedly collected at 12 sites where treatments were designated as brood or

non-brood (n=6) or as feedline or middle (n=6). Litter collected on d0, 28, and 35 was analyzed for N, P, K, S, moisture, whereas microbiome sequencing was performed on all samples (d0, 7, 14, 21, 28, 31, 35). Litter quality data were analyzed by day in JMP 15 using one-way ANOVA with means separated using Tukey's HSD ($P \leq 0.05$). Microbiome sequencing was performed on an Illumina MiSeq and analyzed using QIIME2 (2022.4). Main effects of diversity and composition were determined significant at $P \leq 0.05$ and pairwise differences being determined at $Q \leq 0.05$. In a longitudinal mixed effect model in QIIME2, relationship between litter quality data and the richness and evenness of the community was assessed. Depending on location, brood or feedline, litter quality was impacted at different times during the study. On d0, the levels of K were lower among non-brood areas and N levels were higher among the feedline ($P < 0.05$). On d28, moisture was higher at the feedline compared to the middle of the house ($P < 0.05$). On d35, brood impacted moisture and N where litter collected from the non-brood location had lower moisture and N levels ($P < 0.05$). Microbiome analyses revealed alpha and beta diversity compositional changes due to brood, location, and time. Using Analyses of Composition of Microbiomes (ANCOM), brood had increased levels of *Escherichia-Shigella* (W=416) and feedline had higher abundance of *Flavobacteriaceae* (W=462). Incorporating litter quality data, evenness was not impacted by any of the quality metrics; however, richness (Shannon entropy) of the microbiome was decreased as moisture increased regardless of brood or location ($P < 0.05$). In the brood and feedline, P was inversely associated with richness, whereas, with the increase of P, richness of the litter from the non-brood and middle locations increased ($P < 0.05$). Overall, extended downtime, brooding, and location influence the microbiome of poultry litter with litter quality directly impacting the richness of the litter microbiome at the brood and feedline locations.

Key Words: microbiome; brooding; litter quality; litter management; extended downtime

175 Split-feeding could potentially improve broiler breeder performance. Rick van Emous^{1*}, ¹Wageningen Livestock Research, Wageningen, Netherlands.

Broiler breeders are typically fed a limited amount of feed in the morning which is rapidly consumed. This strategy results in an inadequate nutrient administration to support eggshell formation, affecting the eggshell quality. Therefore, an experiment was conducted to study the effects of feeding either more meals or split-feeding diets (specific morning and afternoon diets) to broiler breeders on reproduction performance and eggshell quality. Ross 308 female breeders were randomly placed in 24 floor pens and housed between 45 and 65 wk of age (WOA) with 30 females and 1 male per pen. The pens were distributed amongst three treatments: 1) Standard breeder diet fed once

a day (100% at 0730 h) (CON), 2) Standard breeder diet fed twice-a-day (50% at 0730 h and 50% at 1600 h) (TAD), and 3) Split-feeding fed, with a specific morning (0730 h) and afternoon (1600 h) diet composition (SF). The SF morning diet contained more energy, protein and phosphorus (P) and less calcium (Ca) than the control and SF afternoon diet. The SF afternoon diet had a lower energy, protein and P and higher Ca content than the control and SF morning diet. Water intake was recorded daily. All eggs per pen were collected daily, graded and recorded. At 52, 59, and 65 WOA, all eggs were opened at day 7 of incubation to determine unfertilized eggs and age of early embryonic mortality. Breaking strength, eggshell thickness, eggshell weight, specific gravity and albumen to yolk ratio were determined at 45, 50, 55, 60 and 65 WOA. Data were analysed using the ANOVA procedure. All birds fed twice a day (TAD and SF) tended towards a lower water intake ($P = 0.055$) and water-to-feed ratio ($P = 0.054$) compared to the CON birds. The SF birds tended to a higher egg production during the entire experimental period compared to the CON birds (74.5% vs. 72.4%; $P = 0.063$), while the TAD birds did not differ from the other treatments. The mortality of the SF birds tended to be lower compared to the TAD birds (5.4% vs. 10.4%; $P = 0.098$), but not to the CON birds. No differences were found for other production characteristics and eggshell quality. The albumen to yolk ratio was higher ($P = 0.022$) for the TAD birds compared to the CON and SF birds. The albumen percentage tended to be higher ($P = 0.060$), while the yolk percentage tended to be lower ($P = 0.069$) in eggs from TAD birds compared to eggs from SF birds, while the eggs from CON birds did not differ. In conclusion, split-feeding has potential to improve reproduction with no effects on eggshell quality and incubation traits. Further research is necessary to improve and optimize split-feeding diets.

Key Words: broiler breeders; production; eggshell quality; split-feeding; twice-a-day feeding

176 Non-digestible nitrogen as formulation predictor of nitrogen excretion in broiler chickens. Tahir Mahmood², Friedrich Rouffineau¹, Angélique Cayzac¹, Yves Mercier^{1*}, ¹Adisseo France S.A.S., Malicorne, France, ²Adisseo Animal Nutrition DMCC, Dubai, United Arab Emirates.

Crude protein (CP) is a common expression of nitrogen (N X 6.25) without a clear distinction between protein and non-protein N as revealed by frequent differences observed with the sum of total amino acids (true protein; TP). Digestible protein (DP) referring to the sum of digestible amino acids is a more accurate estimate of raw materials protein quality. However, depending on the raw material, difference exists between CP and DP and this difference can be referred as non-digestible N (NDN) which presumably will be lost in excreta contributing to N emission. Based on these attributes of the broiler feed formula, we hypothesized that NDN criteria would better predict N excretion irrespective

of feed CP level in broiler feed. To test this concept, we formulated isocaloric (ME, 2950 kcal/kg) experimental diets balanced in essential amino acids: at high (H, 21.5%) and low (L, 18.8%) CP levels, and three levels of NDN leading to four diets: H3.9; H3.01; L3.01 and L2.6. After 0-10 d on a common starter diet, 120 birds were randomly allotted to 1 of the 4 dietary treatments with 30 replicates of one bird for each treatment and experimental diets were fed from 11-22 d. A digestibility assay was conducted to measure N intake, N retention (N_{ret}), total N, uric acid-N (UA-N), and NH_3 -N excretion. The data was subjected to ANOVA and means were compared by Tukey's test. Further, stepwise regression analyses were run to evaluate the impact of dietary CP, TP, DP, NDN, feed intake, and their respective interactions if any, on different response variables. Decreasing CP resulted in lower N intake without any effect of NDN level ($P>0.1$), whereas higher NDN induced higher N intake at high CP level ($P<0.05$). NDN level showed a significant effect ($P<0.05$) on total N excretion irrespective of the CP level ($H3.9>H3.01=L3.01>L2.6$). In terms of percentage, H3.9 had the lowest N_{ret} while L2.6 had the highest N_{ret} ($P<0.05$). Interestingly, the diets varied significantly for UA-N excretion. High NDN at both CP levels led to high UA-N excretion ($P<0.05$). Likewise, NH_3 -N excretion was highest in H3.9 and lowest in L2.6 group ($P<0.05$). The stepwise regression analysis indicated that total N and UA-N excretion were increased by the FI, NDN and DP without interaction, while CP and TP had non-significant impact. Nonetheless, only FI and NDN impacted the faecal N excretion. The scaled estimates in the regression model showed that the effect of NDN was always proportionally higher than dietary CP, TP and DP (the main effect being FI). Taken together, these results indicate that the calculated NDN of the feed formula should be of interest to accurately predict broiler nitrogen excretion leading to more sustainable feed formulation.

Key Words: Broilers; Crude protein; Non-digestible nitrogen; Nitrogen emission

177 Comparative study between a saponin-based solution and conventional solutions for the management of coccidiosis in broiler chickens: a case study under commercial conditions. Mohammed el Amine Benarbia^{1*}, ² Pierre Chicoteau^{1,2}, ¹Nor Feed, Angers, France, ²Joint Lab ANR FeedInTech (FIT: SONAS/Nor-Feed), Beaucauzé, France.

Today, poultry producers are confronted with the need to make choices in coccidiosis management in order to meet the different challenges. A large number of solutions are proposed. However, most of these alternatives to the conventional solutions have only evidence based on *in vitro* models and/or *in vivo* models. Even if data from these models are useful, there is a need to generate data for these alternative solutions from commercial farming conditions. The objective of this study was to generate data for a natural solution based on saponins, Norponin XO2®. The study

was conducted over 4 cycles (35 days) using 4 buildings with a capacity of 15 000 birds each (A, B, C and D). Birds in building A and B were supplemented with Saponin-based solution (Norponin XO2® - 250ppm) during the 4 cycles from d1 to D35. Birds in buildings C and D were supplemented with either Clopidol - 125 ppm (Cycle 1), Lasalocid 75ppm (Cycle 2 and 3), or Salinomycin 60ppm (Cycle 4). Zootechnical performances were monitored and European Poultry Efficiency Factor was calculated for each building and cycle. Obtained data were recorded. Statistical analysis was performed by Fischer t-test using GraphPad® prism 7 software. Statistical significance was considered at $P < 0.05$. No cases of coccidiosis were reported in any of the houses. This shows that the disease was well managed with both the conventional and the saponin-based solutions (Norponin XO2®). No differences were observed in the growth parameters between the chickens supplemented with the saponin-based solution and the chickens supplemented with the conventional solutions (ADG, FCR). However, a significant reduction in mortality was observed in the birds supplemented with the saponin-based solution compared to the others. This was reflected in the EPEF which was higher in birds supplemented with saponin-based solution (Norponin XO2) but not statistically significant. This study demonstrated, based on growth performance and health parameters, that under commercial conditions, supplementation with the saponin-based solution (Norponin XO2) is as efficient as conventional solutions for the management of coccidiosis in broiler chickens. In addition, the mortality rate was reduced in chickens supplemented with saponin-based solution (Norponin XO2). This result has already been described for saponin-based solution (Norponin XO2) using other models. Based on these results, it can be concluded that saponin-based solution (Norponin XO2) is a suitable solution that can be used by professionals to gain agility in the management of coccidiosis.

Key Words: coccidiosis; Eimeria; Broiler chicken; Saponins; Management

178 Comparative effect of dietary inclusion of synbiotic and phytobiotic on production performance, immunity, nutrient digestibility and behavioral response of broiler. Asad Khan^{1*}, Ghulam Hashmi¹, Muhammad Arshad¹, ¹Riphah College of Veterinary Sciences, Lahore, Pakistan.

The study was designed to investigate the comparative effect of dietary inclusion of synbiotic and phytobiotic on production performance, immunity, nutrient digestibility, and behavioral response of broiler. For this purpose, total 375 day-old Hubbard male broiler chicks were reared in a semi-control experimental farm under the following dietary treatments; T1 was considered as the control group (CG) without any additive, T2 diet contain Phytobiotic extract at 5ppm, T3 diet containing probiotics (contained spores of *Bacillus subtilis* @cfu/g) @ 1 g /100Kg, T4 diet containing prebiotic (a refined yeast-based mannan

oligosaccharides preparation) at 2.5g /100Kg, and T5 diet containing 20 mg Lincomycine per Kg. Result of present study revealed that phytobiotic group showed significantly better eating behavior ($P<0.05$) as compared to other groups. Standing behavior revealed that birds belong to probiotics or prebiotics groups showed significantly better performance, as well as walking behavior found positive in prebiotic group. Birds found more aggressive in antibiotics group. Phytobiotics extract and probiotics showed significantly higher body weight gain ($P<0.05$) on 14 and 28 day, and increased feed consumption 14 day as compared to other experimental group. Moreover, FCR of the birds which were fed diets containing phytobiotic extract was found to be significantly better ($P<0.05$) on 14th and 28th day as compared to other experimental groups. Birds belong to herbal extract (HE) formula found higher

($P<0.05$) carcass proportion, breast weight, thigh weight, and gible weight as compared to birds of other experimental groups. However, abdominal fat found to be lower herbal extract group. The Newcastle Disease titer was found higher in HE group ($P<0.05$), as compared to control, prebiotic and antibiotic groups, however, statistically higher from probiotic group. However, Infectious Bursal Disease titer and total serum protein was found non significantly different among all experimental groups. Protein digestibility found significantly higher in HE and probiotic group. Overall, herbal extract expressed positive impact on behavior, performance, antibodies titer, and protein digestibility, and found suitable alternative for antibiotics in broiler diet.

Key Words: broiler; Synbiotic; Phytobiotics

Metabolism and Nutrition, Vitamins and Minerals

179 Determination of the standardized ileal digestible calcium requirement of Ross 308 broilers from hatch to day 14 post-hatch. Carrie Walk^{1*}, Pauline Jenn², Anaëlle Tschambser², Ivan Gaytan-Perez², Raffaella Aureli², ¹DSM Nutritional Products, Heanor, United Kingdom, ²DSM Nutritional Products, Village-Neuf, France.

An experiment was conducted to determine the standardized ileal digestible (SID) calcium requirement of fast-growing broilers from hatch to day 14 post-hatch. 360 Ross 308 male broilers were obtained at day of hatch and allocated to 1 of 5 treatments in battery cages. There were 6 birds/cage and 12 cages/treatment. Four treatments were formulated using SID Ca to contain 0.60, 0.46, 0.32 or 0.18% SID Ca. The final treatment was formulated using total Ca at 0.90% and was expected to meet or exceed all nutrient requirements. This treatment was the reference diet for comparison and validation of the SID Ca diets. Birds and feed were weighed at placement and on day 14. Tibias and ileal contents were obtained on day 14 and excreta was collected/cage. Data were analyzed using JMP and requirements were estimated using 3 different non-linear models. Increasing the SID Ca content in the diet from 0.18 to 0.60% increased (quadratic, $P < 0.05$) BW gain and mortality corrected feed conversion ratio (mFCR). The estimated SID Ca requirement to optimize gain or mFCR was between 0.39 to 0.52%. Tibia ash percent and weight increased (quadratic, $P < 0.05$) as SID Ca content in the diet increased. The estimated SID Ca requirement was between 0.32 and 0.58% using tibia ash. Finally, P utilization was improved ($P < 0.05$) in birds fed SID Ca compared with birds fed the reference diet. In conclusion, the estimated SID Ca requirement of fast-growing broilers from hatch to day 14 was 0.47%. This corresponds to a SID Ca to available P ratio of 1.05:1 or a SID Ca to digestible P ratio of 1.18:1. These results are similar to previously published data evaluating the SID Ca requirement of broilers from hatch to day 10.

Key Words: broiler; tibia ash; performance; digestible calcium

180 Effect of chelated zinc, copper and manganese on growth performance and gut health of broilers subjected to an *Eimeria* challenge Juxing Chen^{1*}, Frances Yan¹, Kyle Cook¹, Robert Buresh¹, Melanie Roux¹, Mercedes Vazquez-Anon¹, Deana Hancock¹, ¹Novus International Inc., St. Charles, Missouri, United States.

With antibiotic growth promoter (AGP) free production increasing, more producers and feed additive companies are evaluating natural approaches to maintaining optimal gut health and bird performance. Trace minerals Zn and Cu play important roles in immune modulation and in modulating gut microbiota, respectively. Therefore, trace minerals could be one of the approaches to improve gut health and performance of broilers under AGP-free production

conditions. A battery cage study was conducted to evaluate the effect of high dose of inorganic trace minerals (ITM) with ZnSO₄, TBCC and MnSO₄ and low dose of mineral methionine hydroxy-analogue chelate (MMHAC) (MINTREX® Zn:Cu:Mn, Novus International, Inc.) on growth performance and gut health of broilers subjected to *Eimeria* challenge. The study consisted of 2 dietary treatments - ITM [ZnSO₄ 110 ppm, tribasic copper chloride (TBCC) 125 ppm, MnSO₄ 120 ppm] and MMHAC (Zn:Cu:Mn 40:30:40 ppm), each with 36 replicate pens of 7 birds per pen in a randomized complete block design. All birds were orally gavaged with a coccidiosis vaccine (mixed species of *Eimeria acervulina*, *Eimeria tenella*, and *Eimeria maxima*) at 1x the recommended dose on d0, 4x the recommended dose on d7 and 16x the recommended dose on d14. Body weight, feed intake, and feed conversion ratio (FCR) were determined on d7, 14, 20 and 28. On d28, one bird per pen was sacrificed to collect jejunal tissue to measure inflammation related gene expression. Data were subjected to one-way ANOVA. In comparison to high dose of ITM, the reduced dose of MMHAC increased ($P=0.018$) body weight by 37 grams on d28, improved ($P<0.05$) cumulative mortality adjusted FCR during all phases with 2.1 points improvement on d28, and reduced jejunal IL17 ($P=0.046$) gene expression on d28. There was no effect on feed intake. In summary, supplementation of the reduced levels of chelated trace minerals MMHAC significantly improved growth performance compared to high levels of ITM with TBCC in broilers subjected to an *Eimeria* challenge.

Key Words: broiler; growth performance; *Eimeria* challenge; Chelated trace minerals; ITM

181 Effect of dietary organic selenium in growth performance, carcass and parts yield, blood metabolites, and breast myopathies in broilers subjected to heat stress. Guilherme Silva Tesser^{1*2}, Nilton Rohloff Junior¹, Cleison De Souza¹, Maressa Cardoso Pereira¹, Raquel Schoffen¹, Cristine Kaufmann¹, Clauber Polese¹, Jessica Starkey², Cinthia Eyng¹, Ricardo Nunes¹, ¹Western Paraná State University, Marechal Cândido Rondon, Paraná, Brazil, ²Auburn University, Auburn, Alabama, United States.

Selenium (Se) has a function related to antioxidant and anti-inflammatory activity and also may reduce myopathies and mitigate negative impacts due to heat stress. The objective of this study was to evaluate the effect of organic Se supplementation in broiler growth performance, carcass and cuts yield, blood and hormonal parameters, and meat quality. One-d-old chicks ($n = 1,000$; Cobb 500) were assigned to 10 replicate pens (20 birds per pen) and fed 1 of 5 dietary treatments (0, 0.13, 0.25, 0.50, and 1.0 mg Se per kg of feed). Birds were reared for 21 d under normal temperature according to breeder recommendations and then subjected to cyclic heat stress (34 °C) from 0900 to

1500 h on d 22 to 42. Growth performance was determined on d 21 and 42. At d 41, 1 broiler per pen was randomly selected and fasted for 6 h prior to blood collection. Plasma samples were analyzed for uric acid (UA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), cholesterol (CHO), creatinine (CRE), gamaglutamyltransferase (GGT), glucose (GLU), lactate dehydrogenase (LDH), total protein (TP), triglycerides (TRI) and creatine phosphokinase (CPK) using commercial kits with a high-performance automatic spectrophotometer. Corticosterone (COR) and thyroxine (T4) hormones were quantified with a monochromator-based microplate reader. At d 42, 4 animals per pen were selected and euthanized to determine carcass and parts yield and to evaluate Wooden Breast (WB) and White Stripes (WS) myopathies. For meat quality analysis, breast muscle samples were used to determine cooking loss (CL), shear force (SF), and water holding capacity (WHC). Thiobarbituric acid reactive substances (TBARS) analysis was performed for lipid peroxidation after 10, 30, and 60 d of storage at -20 °C. as a 1-way ANOVA using SAS PROC GLM with orthogonal polynomials and a regression analysis was performed when a linear or quadratic effect with a 95% confidence interval was observed. No treatment effect on growth performance, carcass and part yields, UA, ALT, AST, ALB, CHO, CRE, GGT, GLU, LDH, TP, CPK, T4, CL, SF, WHC, or TBARS was observed ($P \leq 0.062$). Interestingly, a linear increase in COR as dietary organic Se supplementation increased was observed ($P = 0.0348$). In addition, at d 42, there were no differences among treatments for incidence or severity of WB and WS ($P = 0.4846$). In conclusion, organic Se supplementation of broilers reared under heat stress did not affect growth performance or meat quality parameters, but increased circulating COR concentrations linearly.

Key Words: antioxidant; broilers; White Striping; Wooden Breast; organic selenium

182 The importance of using a highly bioavailable dietary selenium source in improving the redox status, gut health, and performance of broilers exposed to hot temperature. Mohammed Hachemi^{1*}, Hua Sun², Ling Zhao², Zi-Jian Xu², Michele De Marco¹, Mickael Briens¹, Xiang-Hua Yan², Lv- Hui Sun², ¹Adisseo, Antony, France, ²Huazhong Agricultural University, Hubei China, China.

As global temperatures rise, managing poultry under hot conditions becomes a challenge. The first effect of high ambient temperature is a reduced feed intake, resulting in performance drag. To alleviate the negative effects of heat stress, highly bioavailable micronutrients should be supplemented into the feed. Selenium (Se) plays a key role in the antioxidant defenses, preventing tissue damage and allowing normal metabolic processes for optimal growth performance. Se supplements exist in inorganic forms, like sodium selenite (SS), and organic forms like seleno-yeasts (SY), or pure organic forms such as hydroxy-

selenomethionine (OH-SeMet). The higher bioavailability of OH-SeMet than SY and SS is well established; however, its effect in broilers under stressful conditions needs to be explored. This study investigated whether OH-SeMet enhances broiler oxidative stress response greater than SS or SY under environmental heat stress. To determine this, day-old male Cobb 500 broilers (12 cages/diet, 9 broilers/cage) were fed a basal diet (0.047 mg Se/kg) supplemented with SS, SY or OH-SeMet at 0.3 mg Se/kg feed for 42 days. Animals were raised at 18 broiler/m² under natural heat stress conditions (average temperature 33.4°C ± 0.7, relative humidity 70.9%). Data were analyzed using one-way ANOVA ($P \leq 0.05$) and means were separated using the Tukey-Kramer test. The Mortality-corrected feed conversion ratio was significantly improved only by OH-SeMet compared to SS (SS:1.74; SY:1.72; OH-SeMet:1.71g/g; $P < 0.05$). OH-SeMet increased the Se concentration in pectoral muscle and jejunum compared to SS ($P < 0.05$) and was higher in pectoral muscle when compared with SY ($P < 0.05$). In the muscle, both organic forms of Se significantly increased the selenoprotein S (SELENOS) and thioredoxin reductase (TXNRD) productions compared to SS ($P < 0.05$), while only OH-SeMet significantly increased selenoprotein W (SELENOW) compared to SY ($P < 0.05$). Selenoprotein N and W (SELENON and SELENOW) production and thioredoxin reductase (TXNRD) activity were the highest in jejunum of OH-SeMet fed birds ($P < 0.05$). OH-SeMet improved total antioxidant capacity compared to SS in muscle and compared to SS and SY in jejunum. OH-SeMet reduced the malondialdehyde and the protein carbonyl in the muscle more than SY ($P < 0.05$). Intestinal morphology indicates that only OH-SeMet results in a higher ($P < 0.05$) villus height to crypt depth ratio than SS in the duodenum and ileum (SS:3.53; SY:3.93; OH-SeMet:4.14 and SS:3.87; SY:4.80; OH-SeMet:4.76, respectively). These results confirm the importance of a high status in Se thanks to OH-SeMet to improve the redox status, intestinal epithelial homeostasis, and growth performance of broilers raised under hot conditions.

Key Words: antioxidant; hydroxy-selenomethionine; Broiler; selenoproteins; Heat stress

183 Inorganic selenium content and speciation in selenium yeasts. Mohammed Hachemi¹, Denise Cardoso^{1*}, Mickael Briens¹, ¹Adisseo France S.A.S., 92160 Antony, France.

Selenium (Se) is an essential trace mineral for animal nutrition largely due to its key roles in the antioxidant system, metabolism and inflammatory modulation. Selenium is typically supplemented into livestock premixes via sodium selenite or sodium selenate (these are known to have low bio-efficacy) or inorganic forms via selenium yeasts (SY) or pure, chemically synthesized hydroxy-selenomethionine (OH-SeMet; these are known to have a higher bio-efficacy). SY products are well known to contain

different Se species and, based on previous analytical methods, were only shown to contain mainly selenomethionine (SeMet), selenocysteine (SeCys), residual selenite (Se^{IV}) and selenate (Se^{VI}); other organic and inorganic Se forms can be characterized but are rarely quantified. Recently, however, Vacchina et al. (2021) developed a new analytical method to quantify elemental Se (Se⁰), an inorganic Se species. This method provides better insight and an update on the proportion of inorganic Se in SY products. Hence, the objective of the study was to quantify the Se⁰ in SY products and assess the proportion of inorganic Se in those products. To accomplish this, the Se composition of 13 fresh commercial samples of SY products, from different suppliers and batches from the same supplier (CNCM I-3060, CNCM I-3399, NCYC R397, NCYC R645, NCYC R646), were assayed for total Se, inorganic Se species (Se^{IV}, Se^{VI} and Se⁰), and organic Se species (SeMet and SeCys). The samples were analyzed in duplicate. Results in terms of total Se were in line with expected Se concentrations for all 13 SY samples. The concentration of Se⁰ (mg/kg \pm uncertainty) and proportion (%) of Se present as Se⁰ were SY1: 206 \pm 2 (9%), SY2: 116 \pm 1 (6%), SY3: 156 \pm 2 (8%), SY4: 73 \pm 9 (4%), SY5: 247 \pm 6 (13%), SY6: 664 \pm 46 (52%), SY7: 641 \pm 67 (30%), SY8: 196 \pm 2 (9%), SY9: 255 \pm 6 (9%), SY10: 345 \pm 6 (11%), SY11: 177 \pm 30 (8%), SY12: 377 \pm 4 (18%), SY13: 139 \pm 3 (7%). The quantification of Se⁰ in SY products, in addition to Se^{IV} and Se^{VI}, indicated an average proportion of inorganic Se of 14.2%, with a range of 4% to 52% for the 13 SY products. The proportion of Se as SeMet ranged between 19% to 72%, with an average of 55.8%, confirming that the variability in SeMet content for SY products is extremely high. The SeCys content was also variable with an average of 3.8%. The Se⁰ quantified in SY is hypothetically from the reduction of selenite used during the yeast fermentation process. In conclusion, advances in the analytical characterization of SY reveal that there can be a significant proportion of inorganic Se in SY products, which explains their variable and lower bio-efficacy compared to the pure organic form of Se (SeMet).

Key Words: speciation; selenium yeasts; elemental selenium; inorganic selenium; selenomethionine

184 BoreOX, a novel proprietary blend of polyphenol extracts bolsters growth performance and antioxidant status of broilers. Bertrand Medina^{1*}, Dana Kumprechtova², Ivan Girard¹, ¹Probiotech International Inc., St-Hyacinthe, Quebec, Canada, ²Probiotic S.R.O., Brno, Czechia.

This last decades, polyphenol rich botanicals content were widely investigated as new antioxidant chain links able to spare and/or strengthen vit.E inclusion. However, these new promising pure or crude polyphenol extracts were very often ranked under *in-vitro* conditions which are still far from representing *in-vivo* ones. Therefore, this study was designed to confirm any pre-defined *in-vitro* vit.E substitution factor of a novel proprietary blend of

polyphenol extracts [BoreOX® (BX500), Probiotech International Inc.]. A total of 1,260 Ross 308 male broilers were allocated to three dietary treatments: Standard with 84, 56 and 50 ppm of vit.E expected in starter (S), grower (G) and finisher (F) feeds, respectively (SVE); Low vit.E with 40, 25 and 25 ppm of vit.E expected in S, G and F feeds (SLE) and SLE diet enriched with 10 and 5 ppm of (BX500) in S and G, F feeds, respectively (SLEBX). The same dietary program was offered *ad libitum* to 6 replicated pens of 70 birds from 0 to 42 days of age with robenidine as anticoccidial agent. Live weights (LW) and feed intakes were recorded on day 14, 28 and 42, as mortality rates. Data were analyzed by an ANOVA single-factor model with treatment as fixed effect. Serum parameters as vit.E, GSH-px and Total Antioxidant Status (TAS) were analyzed at d14 and 42 (2 birds/pen/d). The dietary vit.E content analysis differed from the designed difference (S: 71.2, 41.4, 37.7; G: 54.7, 29.2, 26.0; F: 47.3, 30.0, 29.8 ppm of vit.E for SVE, SLE and SLEBX, respectively). This failure might explain similar growth performances between SVE and SLE groups but the lower vit.E content remained sufficient to decrease serum vit.E level at d42 (30.6 vs 26.1 μ mol/L, $P < 0.05$ for SVE and SLE, respectively). Regarding polyphenols inclusion, final LW of BX500 supplemented birds was significantly heavier compared to other groups (3173b vs. 3194b vs. 3270a, a, b $P < 0.05$ for SVE, SLE and SLEBX, respectively). Moreover, overall FCR ratio was also improved in polyphenols supplemented group compared to other ones (1.66b vs. 1.63ab vs. 1.59a, a, b $P < 0.05$ for SVE, SLE and SLEBX, respectively). Mortality rates did not differ significantly between treatments. In addition, at d14, serum GSH-px was higher for SLEBX compared to SLE (429.1 vs 367.6 μ kat/L, $P < 0.05$) and at d42 serum vit.E level was the highest for SLEBX (30.6b vs. 26.1a vs. 33.2c μ mol/L, a, b, c $P < 0.05$ for SVE, SLE and SLEBX, respectively). According to these data, the low inclusion levels of this novel blend of polyphenol extracts appeared to be able to restore or even exceed potential impairs due to the experimental decrease of dietary vit.E content. Further *in-vivo* trials should be required to confirm the vit.E substitution factor.

Key Words: broilers; growth performance; tocopherol; polyphenols; serum vitamin E

185 Effects of the *in ovo* administration of the Marek's Disease vaccine alone or in combination with the *in ovo* and dietary administration of supplemental 25-hydroxyvitamin D₃ in Ross 708 broilers. II. Immunological and inflammatory responses, and small intestine histomorphology. Saman Fatemi^{1*}, Ayoub Mousstaaid¹, Katie Elliott¹, April Levy², Ishab Poudel¹, Sabin Poudel¹, David Peebles¹, ¹Mississippi State University, Starkville, Mississippi, United States, ²DSM Nutritional Products, Ankeny, Iowa, United States.

The separate administration of dietary or *in ovo*-injected 25-hydroxyvitamin D₃ (25OHD₃) has been shown to

improve the inflammatory reaction as well as the small intestine morphology of broilers. However, the possible synergistic effects of both dietary and *in ovo* 25OHD₃ sources in conjunction with the Marek's Disease vaccine (MDV) have not been previously reported. The objectives of this study were to determine effects of the MDV alone or in combination with the *in ovo* and dietary administration of 25OHD₃ on the systemic immune response and small intestine morphology of Ross 708 broilers. Live embryonated hatching eggs were randomly assigned to one of the following 4 *in ovo* injection treatments at 18 d of incubation. 1) Non-injected; 2) MDV alone; or MDV containing either 3) 1.2 (Hy-D-1.2), or 4) 2.4 (Hy-D-2.4) µg of 25OHD₃. Hatchlings were assigned to the one of the following posthatch dietary treatments: 1) commercial diet containing 250 IU of vitamin D₃ /kg of feed (control); or 2) commercial diet supplemented with an additional 2,760 IU of 25OHD₃ /kg of feed (HyD- diet). In the grow out period, each of the 8 treatments was replicated in 6 pens (48 total pens), each containing 14 chicks. At 14 and 40 d of age (doa), blood samples were collected from 1 bird per pen for determination of serum IgG, nitric oxide, and α-1-acid glycoprotein (AGP). Also from the same bird, villus length (VL), crypt depth, villus length to crypt depth ratio (RVC), and villus surface area in the duodenum, jejunum, and ileum were determined. A randomized

complete block experimental design was used and all data were analyzed using a two-way ANOVA. No significant dietary x *in ovo* treatment interactions were observed for any of the variables examined. At 14 doa, birds fed Hy-D diets had lower (P=0.024) serum AGP levels compared to birds fed commercial-fed diets birds. Additionally, *in ovo* injection of either Hy-D-1.2 or Hy-D-2.4 resulted in a decrease (P=0.036) in serum AGP in comparison to the MDV alone and non-injected treatment groups at 40 doa. At 14 and 40 doa, a higher jejunal RVC (P=0.004) was observed in birds that were *in ovo*-injected with Hy-D-1.2 as compared to the MDV alone and non-injected treatment groups, and dietary Hy-D diets increased VL in the duodenum at 14 doa (P=0.001) and in the duodenum and jejunum at 14 and 40 doa (P=0.003) in comparison to the commercial diet. In conclusion, dietary or *in ovo* administration of 25OHD₃ depressed the inflammatory reaction and improved the small intestine absorptive capability of broilers was exemplified by the suppression of serum AGP levels and improvements in intestinal villus morphology.

Key Words: *in ovo* injection; 25-hydroxycholecalciferol; Marek's Disease vaccine; inflammatory responses; small intestine morphology

Metabolism and Nutrition, Feed Additives II

186 Effects of broiler breeder age and addition of enzymatically treated yeast to diets fed to younger breeder chicks on growth performance and apparent metabolizable energy. Anderson Maina^{1*}, Aizwarya Thanabalan¹, Jessica Gasarabwe¹, Mohsen Mohammadigheisar¹, Hagen Schulze², Elijah Kiarie¹, ¹University of Guelph, Guelph, Ontario, Canada, ²Livalta, Peterborough, United Kingdom.

Older broiler breeders hatch heavier and better-performing chicks than younger breeders. We assessed whether feeding enzymatically treated yeast (HY40) to young broiler breeder chicks (YBC) can bolster growth to the level of older broiler breeder chicks (OBC). Fertile eggs: 2,250 (56.5±3.4g) from 30-week-old and 550 (64.2±4.2 g) from 47-week-old Ross 708 broiler breeders were hatched. Based on hatch body weight (BW), chicks were placed in floor pens (8 pens, 44 chicks/pen) for growth performance and metabolism cages (36 cages, five chicks/cage) for apparent retention (AR) of nutrients and metabolizable energy (AME). Five corn and soybean meal-based diets were formulated to contain 0, 0.05, 0.10, 0.20, and 0.40% HY40 for a 3-phase feeding program: starter: d 0-10, grower: d 11-24, and finisher; d 25-42. Grower phase diets also contained 0.3% TiO₂ indigestible marker. The diets were allocated within YBC in a completely randomized block design (n=8 for pens; n=6 for cages). The OBC was fed 0% HY40 diets. Feed and water were provided freely; BW and feed intake were monitored, and excreta samples were collected on d 17-21 for AME measurements. Data were statistically analyzed using the GLM procedures of SAS, with treatment as a fixed factor and LSmeans separated using Tukey test and curve response HY40 inclusion in YBC birds. Except for overall FCR, which was similar, OBC exhibited superior performance indices than YBC at 0% HY40, including lower numerical mortalities and heavier breasts ($P=0.001$). The AR of crude protein, dry matter and AME was higher in OBC than in 0% HY40 YBC ($P<0.003$). The YBC 0% HY40 had lower ($P<0.05$) final BW than YBC fed 0.05, 0.1, 0.2 and 0.4% HY40. Overall, in YBC, feed intake was higher in 0% YBC HY40 than in 0.2 and 0.4% rates (quadratic, $P=0.05$). Breast yield was lower in 0% HY40 YBC than in 0.05, 0.1, 0.2 and 0.4% inclusion rates, with 0.2 and 0.4% having the heaviest but similar weights ($P=0.001$); however, HY40 increased breast weight linearly ($P=0.019$) and quadratically ($P=0.037$) in YBC. The AR of dry matter, crude protein and AME was higher ($P=0.01$) for YB fed $\geq 0.05\%$ HY40 relative to YBC 0%HY40. There were no YBC on mortalities ($P=0.091$). In conclusion, OBC showed higher growth driven by initial BW and feed intake than YBC not fed HY40. Dose-response analysis suggested optimal performance in YBC was achieved at 0.2% HY40 inclusion. However, nutrient utilization linearly increased with the inclusion of HY40, suggesting enhanced intestinal function at higher yeast supplementation.

Key Words: broiler chicken; growth performance; nutrient retention; broiler breeder age; enzymatically treated yeast

187 Comparison of a standard male diet to a docosahexaenoic acid (DHA) supplemented male diet on rooster semen quality and reproductive performance. Kelly Sweeney¹, Luis Avila¹, Jeanna Wilson^{1*}, ¹University of Georgia, Athens, Georgia, United States.

In the US, broiler breeder males are typically fed a female diet out of convenience, but some speculate that roosters have specific dietary requirements not met by a hen formulation. In this study, a basal diet with 2720 kcal/kg energy, 12 % crude protein, and 0.74 % calcium was mixed according to primary breeder guides specific to the roosters needs. The basal was split to mix a male control (MC) diet containing no treatment product and the male DHA (MD) diet containing 0.28 % DHA (docosahexaenoic acid). DHA is an omega-3 fatty acid proven to improve soft tissue development like brain tissue and testes and other omega-3 fatty acids have been proven to improve semen quality. Due to organoleptic problems, cost, and product stability using an alternative to fish oil that is equally as effective is a thriving area of research. The objective of this study was focused on evaluating the dietary inclusion of DHA to improve rooster semen production and quality when compared to a traditional male diet. The treatment groups were fed equal amounts of feed from 16 to 65 wk. A total of 80 Ross Yield Plus roosters were assigned to each dietary treatment (8 replicates, 10 roosters per replicate). During the production phase, approximately 25 to 30 % of males in each treatment were weighed weekly, with groups rotating over time. Percentage of roosters producing semen, semen volume, sperm concentration, and sperm mobility were measured every 5 wk from 25 to 65 wk. Individually caged Ross 708 hens were artificial inseminated with 0.05 mL pooled and diluted semen from a replicate group of males (11-12 hen/rep with 8 rep/trt). Semen was diluted with Avian Buffer to 7.5×10^7 sperm. Eggs were collected for 14 d post-insemination and incubated for a fertility analysis. Data were analyzed by SAS 9.4 SLICE and means separated by LSD (significance level $P \leq 0.05$). The MD diet had no significant influence on body weight, semen volume or 65 wk testicle weight. Early in life semen production was significantly impacted ($P=0.0007$) at 25 wk with a larger percentage of males on the MD diet (95.0 %) being in semen production than those males on the MC diet (88.1%) with 65 wk production at 95.5% and 93.3%, respectively ($P=0.131$). Sperm mobility was significantly impacted from at wk 33, 38, 48, and 62 ($P<0.05$) with the males fed the MD diet having greater sperm mobility (0.412-0.482 absorbance) than those on the MC diet (0.289-0.369 absorbance). The dietary supplement, DHA had a significant impact on overall fertility through 62 wk ($P<0.0001$) with the males fed the MD diet having 7%

greater fertility than those on the MC diet. DHA has proven to have positive impacts on semen quality and lifelong fertility in broiler breeder males.

Key Words: Fertility; Rooster Diet; Semen Quality ; Omega 3 Fatty Acid; Semen Production

188 Growth performance response of Cobb 500 male broiler chickens to supplementation of a gluconeogenic feed additive in corn-soybean meal diet with reduced energy content. Iyabo Oluseyifunmi^{1*}, Oluyinka Olukosi¹, ¹*University of Georgia, Athens, Georgia, United States.*

In a 42-d study, 432 Cobb 500 male broiler chicks at zero-day old were used to investigate the effects of supplementing broiler diets with a gluconeogenic substrates additive (GA) on growth performance and jejunal histomorphology of birds. The birds were allocated to three dietary treatments in a randomized complete block design. The treatments consisted of a nutrient-adequate positive control (PC) diet, a negative control (NC) diet, and NC + GA. Each treatment had 12 replicates with 12 birds per replicate. The NC diet had 50 kcal/kg lower metabolizable energy (AME) than the PC diet. The lower AME in NC diet was obtained by removal of 10 g/kg soybean oil relative to the PC diet. Birds and feed were weighed on d 0, 14, 28, and 42 corresponding to the starter, grower and finisher phases. On d 42, after euthanasia, a 5-cm jejunal section was obtained from two randomly selected birds per pen for histomorphological study. All the data were analyzed using MIXED procedure of SAS with block as random factor and diets as fixed factor. There were no significant treatment effects on body weight of the birds throughout the experiment. Compared to the NC diet, GA supplementation resulted in greater gain:feed ($P < 0.05$) in the starter phase (d 0 to 14), tended ($P < 0.10$) to increase gain:feed during the starter plus grower phases (d 0 to 28), and the finisher (d 28 to 42) phase. In the overall phase (d 0 to 42), GA supplementation significantly ($P < 0.05$) increased the gain:feed compared with NC. Villi height tended to be lower ($P = 0.066$) for birds receiving NC and NC+GA diets compared with those receiving the PC diet. It can be concluded that the supplementation of the gluconeogenic additive helped to recover the loss in efficiency of feed utilization caused by reduction in metabolizable energy content of the broiler diets in the broilers used in the current experiment.

Key Words: growth performance; zinc; histomorphology; choline; Gluconeogenic substrates

189 Effect of methionine and trace mineral supplementation on growth performance of broilers under coccidiosis challenge. Frances Yan^{1*}, Vivek Kuttappan¹, Juxing Chen¹, Deana Hancock¹, Mercedes Vazquez-Anon¹, ¹*Novus International, St. Charles, Missouri, United States.*

Coccidiosis is a major challenge to the poultry production causing economic loss to the broiler industry. Vaccinations are available to effectively control coccidiosis. However, birds undergo a few cycles of coccidia infection and could incur performance loss before they develop immunity. The objective of the present study was to evaluate the effect of methionine and trace mineral supplementation on growth performance of broilers under coccidiosis challenge. In a battery cage trial, a total of 720 male broilers were assigned to 9 treatments with 8 replicates/treatment, 10 birds/replicate in a randomized complete block design. The experiment consisted of 2*2*2 factorial + 1 arrangement with main factors as methionine source (DL methionine vs 2-hydroxy-4-(methylthio)-butanoic acid (HMTBa)), total sulfur amino acid (TSAA) level (low vs high; $\pm 5\%$ of recommended level), and trace mineral Zn, Cu, and Mn in the form of sulfates (80:20:100 ppm) vs mineral methionine hydroxy analog chelate (MMHAC; MINTREX® 40:10:50 ppm). In addition, a no challenge control (NoCHAL) was included with HMTBa, High TSAA, and MMHAC in the diet. Birds received corn-soybean meal basal diet along with the respective treatments throughout the 27 days of grow out. All treatments except NoCHAL were orally gavaged with a live coccidiosis vaccine (ADVENT®, containing *E. acervulina*, *E. maxima*, and *E. tenella*) on 0, 7, and 14d at 1X, 4X, and 16X doses, respectively. Data were subjected to one-way and three-way ANOVA and means were separated by Fisher's protected LSD test with significance at $P < 0.05$. Factorial analysis revealed the main effects ($P < 0.05$) of TSAA level and trace minerals, but no difference ($P > 0.05$) in methionine source. Birds fed diets with the high TSAA level showed higher ($P < 0.05$) body weight and cumulative feed intake at 27d, although there was no effect ($P > 0.05$) on feed conversion ratio (FCR) compared to low TSAA level. Birds fed with MMHAC showed improved ($P < 0.05$) body weight, FCR, and feed intake compared to sulfates at 27d. The MMHAC feed birds showed improvement ($P < 0.05$) in period FCR during recovery phase d20-27 compared to sulfates. At the high TSAA level with DL methionine or HMTBa supplementation, MMHAC under challenge had similar ($P > 0.05$) 27d body weight to NoCHAL, whereas the challenged sulfate birds had significantly lower 27d body weight than NoCHAL ($P < 0.05$). In conclusion, dietary inclusion of high TSAA level or MMHAC could improve performance during coccidiosis challenge. However, the two sources of methionine tested showed no differences in the effect on performance.

Key Words: broilers; coccidiosis; methionine; trace mineral; vaccination

190 Effect of a proprietary butyrate product on growth performance and carcass yield of Ross 708 broilers. Chris Rude^{1*}, Shameer Rasheed¹, Kristy Dorton¹, Mahmoud Masadeh¹, Mark LaVorgna¹, ¹*Devenish Nutrition, Fairmont, Minnesota, United States.*

Two studies were conducted to determine the effect of a proprietary butyrate product (UltraGuard Quattro (UGQ), Devenish Nutrition, Fairmont, MN) on growth performance and carcass yield of Ross 708 broilers. In each study, one day old Ross 708 broiler chicks (n = 900; as hatched) were randomly allocated to two treatment groups of 9 pens per treatment (50 birds per pen; 0.10 m²/bird). Each study included four dietary phases: starter (d 0 to 16), grower (d 17 to 31), finisher (d 32 to 44) and withdrawal (d 45 to 57). Birds were fed a corn-soy based pelleted diet, which contained the study treatments. Each study involved two treatment groups: Control (basal diet, no test product) or UGQ at 0.25 g/kg in the starter, grower, and finisher diets. No UGQ was added to the withdrawal diet. A coccidiostat (Zoalene 0.5 g/kg) was included in the starter, grower, and finisher diets to control coccidiosis. Bodyweight and feed consumption were measured at d 0, 16, 31, 45, and 56 (Study 2) or 57 (Study 1) and weight gain and feed conversion were determined. In Study 2, a subset of male broilers from each treatment were processed for carcass yield on d 58. Data was analyzed as a one-way ANOVA using the Mixed procedure of SAS 9.4 (2018) with pen as the experimental unit. Means were separated using Fisher's protected least significant difference. Differences were considered significant at $P \leq 0.05$ and a trend at $0.05 < P \leq 0.10$. In Study 1, broilers supplemented with UGQ had better ($P \leq 0.001$) body weight at d 16 ($P = 0.0001$; 0.57 vs 0.54 kg), 31 ($P < 0.0001$; 1.81 vs 1.89 kg), and 57 ($P = 0.04$; 4.34 vs 4.45 kg) compared to broilers in the control group. Similarly, broilers supplemented with UGQ had better feed conversion from d 0 to 16 ($P = 0.004$; 1.124 vs 1.151) and d 0 to 31 ($P = 0.01$; 1.363 vs 1.381) and better overall (d 0 to 57) body weight-adjusted feed conversion ($P = 0.05$; 1.744 vs 1.771) compared to broilers in the control group. In Study 2, body weight and weight gain were not significantly affected by treatment. However, broilers supplemented with UGQ had better feed conversion from d 0 to 16 ($P = 0.004$; 1.054 vs 1.067), d 32 to 45 ($P = 0.0002$; 1.752 vs 1.812), and d 0 to 45 ($P = 0.01$; 1.482 vs 1.504). Additionally, broilers supplemented with UGQ tended to have a better percentage of post evisceration carcass yield ($P = 0.06$; 78.61% vs 77.62%). Because of the improvement in growth performance, supplementation of UGQ can help achieve feed cost savings per unit of live weight produced. Overall, the results show that supplementation of UGQ improves body weight, feed conversion, and carcass yield.

Key Words: broiler; butyrate; carcass yield; growth performance

191 Prevention of coccidiosis and necrotic enteritis in broilers by dietary supplementation of two proprietary plant-based products. David Diez¹, Sudipto Haldar², Anna Tesouro¹, Amrita Dhara², Felipe Mendy^{3*} ¹Biovet S.A., Constanti, Tarragona, Spain, ²Agrivet Consultancy P Ltd., Kolkata, India, ³IFTA USA INC., Durham, North Carolina, United States.

Coccidiosis and necrotic enteritis (NE) are major poultry diseases caused by *Eimeria* and *Clostridium perfringens* (CP) respectively. Mixed infections are common. The efficacy of two proprietary feed additives to prevent a mixed challenge of *Eimeria* and CP was evaluated in experimentally challenged broilers raised for 42 days. The feed additives designed by Biovet S.A., Alquernat[®] Coneb (AC) and Alquermold[®] Natural (AN), based on a combination of phenolic compounds derived from plant extracts, were administered continuously at 0.5 kg/t each. AC stimulates the activity of the gut's immune system to eliminate *Eimeria* and AN is a strong microbicidal against CP that disrupts the integrity of the bacterial membrane. 640 one-day-old broilers were distributed into 8 treatment groups with 8 replicates each. The non-supplemented treatments were an uninfected untreated control (NC); an untreated *Eimeria*-infected group (PC1) and an untreated *Eimeria* and CP infected group (PC2). The rest of the groups were NC, PC1 or PC2 supplemented with either AC alone or AC and AN together; NC+AC, NC+AC+AN, PC1+AC, PC2+AC, and PC2+AC+AN. *Eimeria* challenge was performed at day 15 of age and CP challenge at days 18-20. Oocysts counts from fresh feces were performed at 7- and 8-days post-infection (dpi). All data were analyzed with MANOVA and $P < 0.05$ was set as statistically significant. Body weight (BW) and feed conversion (FCR) were worse ($P < 0.05$) in PC1 (2835.5 g and 1.605) and PC2 (2819.7 g and 1.620) compared to NC (3063.7 g and 1.531). Regarding uninfected groups, AC alone (NC+AC 3103 g and 1.509) and AC+AN (NC+AC+AN 3131.3 g and 1.492) improved BW and FCR compared to NC, PC1 and PC2 ($P < 0.05$). Regarding challenged groups, AC alone improved FCR (PC1+AC 1.567) compared to PC1 ($P < 0.05$); however, the improvement was not significant compared to PC2 (PC2+AC 1.612). All supplemented groups obtained lower oocyst counts (average: 52.76/g $\times 10^3$) than PC1 and PC2 (average: 215.975/g $\times 10^3$) ($P > 0.05$). Duodenum, jejunum and ileum tissue samples collected 10 dpi were analyzed for gene expression for NC, PC1 and PC1+AC groups. Positive fold change in PCR ($P > 0.05$) was observed for IL-18 gene expression in PC1+AC (0.0857), compared to PC1 (0.0072), meaning AC stimulated the IL-18 production, an inducer of cell mediated immunity associated in Th1 responses to eliminate intracellular parasites like *Eimeria*. In conclusion, AC was highly effective against coccidiosis and increased the expression of cytokines related to Th1 immune response. However, the combination of AC with AN is a better option when coccidiosis is complicated with NE. Besides, AC and AN do not leave residues, nor create resistances.

Key Words: Clostridium; Broilers; Necrotic Enteritis; Coccidiosis; Gut Health

192 Effect of a protease alone or in combination with a precision biotic in broiler chickens under a necrotic enteritis challenge. Shelby Corray^{1*}, April Levy¹, Cristiano

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The objective of the present study was to evaluate the effect of a novel protease (PRO) alone or in combination with a precision biotic (PB) on the growth performance, lesion scores, and mortality of broiler chickens under a necrotic enteritis (NE) challenge model. One-day-old broiler chicks were placed in a completely randomized block design in a 2 x 2 factorial arrangement, with 8 replicates/treatment, and 25 birds/replicate. The treatments consisted of a non-supplemented group (CON), supplementation with 50 ppm of PRO, supplementation with 700 ppm of PB, or supplementation with 50 ppm of PRO plus 700 ppm of PB (PRO+PB). All the birds were vaccinated against coccidiosis at the hatchery (Coccivac-B52). Additionally, all the birds were submitted to a challenge consisting of 5,000 oocysts of *Eimeria maxima* given via feed on d14, and *Clostridium perfringens* strain #6 via feed on d19, 20, and 21. On d21, 2 birds/pen were euthanized and scored for NE lesions, and growth performance was evaluated on days 14, 21, 35 and 42. The data were submitted to two-way ANOVA ($P \leq 0.05$), and LSMeans Tukey was used to separate the means in case of interaction. Non-parametric data were submitted to Shash transformation using JMP 15. On day 14, it was observed that PRO supplementation improved FCR ($P=0.01$). On day 21, an interaction ($P=0.002$) of PRO by PB was observed, wherein the supplementation of PRO and PB, alone or combined, improved the FCR of the birds. On day 35, there was no interaction between both factors, but PRO improved ($P = 0.002$) the FCR by 2.8%, and PB by 4.6% ($P < 0.0001$). On day 42, an interaction was observed for FCR ($P < 0.0001$) and body weight (BW; $P=0.04$). The supplementation of PRO and PB, alone or combined, improved the FCR and BW of the birds. Although not different, on day 42, the combination of PRO+PB improved the BW by 58 and 77 g, and the FCR by 3.7 and 2.3 points when compared to the supplementation of single PRO and PB, respectively. No statistical difference ($P > 0.05$) was observed on mortality. The supplementation of both PRO and PB, alone or in combination, reduced the average ($P=0.03$), and the frequency ($P < 0.05$) of lesions characteristic of NE. This data suggests that the supplementation of PRO and PB tends to be a beneficial strategy to mitigate the negative effect of NE and supports the integrity of the intestinal lining in broiler chickens.

Key Words: broiler; necrotic enteritis; microbiome; protease; precision biotics

193 A novel model of low-grade chronic intestinal inflammation chicken under real farming conditions. Alireza khadem^{1 2} Christos Gougoulas^{2*}, ¹University of Gent, Merelbeke, Belgium, ²Innovad Group, Antwerp, Belgium.

Modern poultry production disorders result in significant economic losses associated with compromised intestinal health, primarily linked to chronic inflammatory status. Due

to the importance, several *in vivo* models of gut failure/dysbiosis have recently been developed, however, the lack of realism of the experimental conditions constitutes a major drawback. Here we validated a novel approach of a broiler chronic, low-grade, intestinal inflammation within a real production setting. Expt. 1 & 2 incorporated two groups i): a high NSP diet (60% Wheat+ 5% rye) without NSPase and coccidiostats, co-located inside a commercial farm (8 cages, n=30 birds/cage), and ii) birds straight from the commercial production fed a low NSP (50% Corn+ 30% soy+ NSPases + coccidiostats). Expt 1 confirmed that the high-NSP diet resulted in increased dysbacteriosis-related gross macroscopic scores compared to the rest of the production (one randomly selected bird per cage vs. 8 randomly selected production birds), which persisted in time albeit to a lesser extent (mean scores at d-21; 5.12 vs. 2.75, $P=0.0026$ and at d-28; 4.38 vs. 2.25, $P=0.0020$; according to the method of De Gussem (2010) which incorporates several inflammation indicators throughout the gut, as well as coccidiosis lesion scores). This picture was significantly worse than the dysbacteriosis produced by a sophisticated but still artificial challenge, under clean experimental conditions (De Meyer et al 2019; comprising of an initial dietary challenge, followed by a course of antibiotics and an experimental infection, made of a cocktail of beneficial and harmful bacteria for three consecutive days and a one-off inoculation of a multi-*Eimeria* mix). In Exp. 2, the growth performance was numerically reduced for the high-NSP diet group vs. the rest of the production at the end of the life cycle (d-35) while a heatwave resulted in elevated mean temperatures inside production i.e. 31.5°C (88.7°F) for ~10-12 hours per day (at D16 and D21 to D29). Interestingly, detailed biomarker monitoring of the high NSP group revealed that, although intestinal oxidative stress related to lipid peroxidation (MDA) was reduced between D28 and D35 by ~30%, the corresponding intestinal inflammatory (IFN- γ) and immune-activation (sIgA) responses were increased (33% and 40% increase, respectively; $P < 0.0001$), exhibiting, thus, a cumulative impact over time, which raises concerns for animal productivity. In conclusion, this novel and realistic chronic gut inflammation model is the sum of several stress points (beyond just the introduced mild dietary challenge) and offers a new powerful tool to the industry, for testing the efficacy of several technologies such as in-feed alternatives to AGPs.

Key Words: biomarkers; novel model; chronic intestinal inflammation; real farming conditions; dysbacteriosis

194 Dietary enzymatically treated yeast and coccidia challenge effects on growth performance, nutrient digestibility, and intestinal health of broiler chickens. Emmanuel Alagbe^{1*}, Hagen Schulze², Olayiwola Adeola¹, ¹Purdue University, West Lafayette, Indiana, United States, ²Livalta, Peterborough, United Kingdom.

The objective of this study was to investigate the effect of enzymatically treated yeast (ETY) on the growth performance, nutrient digestibility, and intestinal health of coccidia-challenged broiler chickens. From d 1 to 14 post hatching, 480 broiler chickens (49.9 ± 3.95 g) were allocated to 3 corn-soybean meal-based diets with increasing concentrations of ETY (0, 1, or 2 g/kg). There were 16 replicate cages and 10 birds/cage. The experiment was designed as a randomized complete block design with body weight (BW) used as a blocking factor. On d 14 post hatching, the birds were combined and re-randomized within each of the 0, 1, or 2 ETY g/kg experimental diets. Following this, the number of birds was reduced to 8 birds/cage with 8 replicate cages of challenge or no-challenge group for each of the 3 diets, resulting in a 2×3 factorial arrangement of treatments. The BW ratio before re-randomization was maintained across all treatments during the reallocation. On d 15 post hatching, the birds in the challenge and no-challenge groups were orally gavaged with 1 mL *Eimeria* oocysts and 1% PBS, respectively. Data were analyzed using the MIXED procedure of SAS, and polynomial contrasts were used to estimate the linear and quadratic effects of ETY. The coccidia challenge (CC) decreased ($P < 0.01$) the BW gain, feed intake, and G:F ratio of broiler chickens from d 14 to 21. Increasing supplementation of dietary ETY improved ($P < 0.05$) the G:F ratio of birds. Also, the CC reduced ($P < 0.01$) the apparent total tract digestibility of dry matter (DM), gross energy (GE), and metabolizable energy (ME). On d 21, dietary ETY linearly increased ($P < 0.01$) the apparent ileal digestibility of DM and GE in broiler chickens. The CC increased ($P < 0.01$) the mRNA gene expression of TNF α , IL-1 β , IL-10, and IL-6. There was a tendency ($P < 0.1$) for ETY to reduce IL-1 β expression in broiler chickens on d 21. Additionally, ETY supplementation increased ($P < 0.05$) the gene expression of occludin in the ceca of broiler chickens but not claudin 1. Serum catalase increased ($P < 0.05$) with increasing supplementation of dietary ETY in broiler chickens on d 21. Dietary ETY linearly increased ($P < 0.05$) the ileal villus height to crypt depth ratio, and ileal goblet cell count and density in broiler chickens. The ileal and excreta oocyst counts decreased ($P < 0.01$) with increasing supplementation of dietary ETY in coccidia-challenged broiler chickens on d 21. In summary, dietary ETY reduced oxidative damage, improved growth performance, enhanced nutrient utilization, and augmented intestinal development in broiler chickens.

Key Words: coccidia; nutrient digestibility; health; yeast; goblet cells

195 Antimicrobial kinetics of a blend of bioactive compounds and its role in maintaining epithelial integrity during a *Clostridium perfringens* challenge on a chicken intestinal cell line. Giulia Giovagnoni^{1*}, Benedetta

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Necrotic enteritis is one of the most common diseases in poultry production worldwide, leading to sub-optimal performance. Aim of this study was to investigate the antimicrobial kinetics of a blend of bioactive compounds against *Clostridium perfringens*, the causative agent of necrotic enteritis. Moreover, the role of this blend in counteracting a bacterial challenge on a chicken intestinal cell line was assessed by measuring epithelial integrity. ATCC® 13124 *Clostridium perfringens* (CP) strain was cultured at 37°C in Reinforced Clostridial Medium in an anaerobic environment. For the time-kill assay, CP at 10^5 CFU/ml was grown without treatments (CTR group) or in presence of either a high dose (HD) or a low dose (LD) of the bioactive compounds blend (botanicals and fatty acids). CP was plated on TSA blood agar every hour, from inoculation (0 hours) for the first 8 hours, and after 24 hours in order to count viable bacteria over time. For the cell culture experiment, chicken intestinal epithelial cells (cIEC) were seeded on 0.4 μ m transwell inserts and allowed to grow in specific expansion media at 37°C and 5% CO₂, until complete polarization. Then, cells were challenged with CP at 10^5 CFU/ml without treatments (CTR+) or with either bacitracin at 2 ppm, the HD or the LD blend (n=6). The CTR- group was used as not-challenged control. The integrity of the epithelial layer was assessed by measuring trans-epithelial electrical resistance (TEER) at 0 hours, 1.5 hours, and 3 hours post-challenge. Data were analyzed with 2-way ANOVA and differences considered significant at $p < 0.05$. In the time-kill assay, both the HD and LD blend allowed a reduction of CP growth compared to the control right after the inoculation and the HD blend completely killed bacteria at 0 hours ($p < 0.0001$). The LD blend significantly decreased CP growth in a more gradual manner and after 4 hours totally killed the microorganism, with no difference from HD blend. During the bacterial challenge on cIEC, CP strongly reduced the TEER at 1.5 hours (-65% than initial value) and even more at 3 hours (-95% than 0 hours). Despite the challenge, the LD group was able to protect the cells preventing TEER drop by 40% and 45% at 1.5 and 3 hours, respectively, compared with the challenged control ($p < 0.0001$). Moreover, bacitracin and HD groups showed a TEER comparable to the not-challenged control at both time-points. The investigated blend of bioactive compounds demonstrated a bactericidal activity against *Clostridium perfringens* and protected epithelial cells during a bacterial *in vitro* challenge in a dose-dependent manner.

Key Words: *Clostridium perfringens*; feed additives; time-kill assay; TEER; bioactive compounds

Metabolism and Nutrition, Enzymes I

196 Understanding the kinetics of jejunal and ileal nitrogen digestibility for successful development of endogenous proteases for animal nutrition. Murtala Umar Faruk^{1*}, L rke Haahr², Franz Roos¹, Guenter Pappenberger¹, Aaron Cowieson¹, ¹DSM Nutritional Products, Kaiseraugst, Switzerland, ²Novozymes, Lyngby, Denmark.

Over the last decade, the use of mono-component proteases to improve nitrogen utilization, reduce feed cost and environmental impact has been receiving enormous attention. The objective of this work was to establish an assay to understand the kinetics of nitrogen digestion, to serve as basis for screening and the development of the second generation of mono-component protease. A total of 71 different proteinases were tested on their ability to improve jejunal and ileal Nitrogen digestibility of 21-day old broiler chickens. A total of 10 experiments were conducted using comparative experimental set-up. A positive control (PC) diet was formulated to include a highly digestible protein source (soy protein concentrate). The negative control diet was formulated to meet the nutrient density of the PC without the addition of the highly digestible protein source. The aim of the diet formulation was to slow down the rate of nitrogen digestion, to allow for protease action to be observed. Animals were fed the NC diet from day-old to 16 days of age. The tested proteases were included into the NC diet and fed from 16 to 21 days old. Jejunal and ileal content were collected to assess apparent jejunal nitrogen digestibility (AJDN) and apparent ileal nitrogen digestibility (AIDN). A linear mixed model with study as random effect was used to evaluate the effect of the enzymes using trial as a random factor. The function lme() in the package nlme in the statistical software R. Overall, across the different trials, AJDN was lower ($56 \pm 8\%$) compared to AIDN ($82 \pm 4\%$). AJDN was equally lower in NC (56%) compared to PC (63%) fed treatments. However, the AIDN was similar between NC ($81 \pm 5\%$) and PC ($82 \pm 4\%$). PC diet had a stronger correlation between AJDN and AIDN ($r^2=0.37$) compared to NC ($r^2=0.19$). Comparing the 71 proteases relative to the NC diet, 49% showed an improved AJDN above zero, while 56% showed positive improvement of AIDN. The rate upon which the enzymes acted differed. Improvement in AJDN did not in all cases resulted to improved AIDN. Approximately 26% of the enzymes had AJDN below the average AJDN but had a terminal AIDN above the average AIDN. These results provide an important basis for selecting optimum protease to meet the poultry digestive physiology. It is known that the ileal section of the gastrointestinal tract contained almost 10 times less peptide transporters compared to the jejunal section. Therefore, an early action of a protease in the proximal section will benefit the animal by making readily available peptides for absorption and reduce the level of undigested protein reaching the hindgut.

Key Words: Digestibility; Nitrogen; Proteases; Screening; Sfericase

197 Performance and nutrient digestibility of broilers supplemented with a novel sfericase protease. Sergio Vieira^{3*}, Jose Otavio Sorbara², Thiago Noetzold¹, Camila Freitas¹, Levy Teixeira², Murtala Umar Faruk², ¹University of Alberta, Edmonton, Alberta, Canada, ²DSM Nutritional Products, Kaiseraugst, Switzerland, ³Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

The aim of these studies was to evaluate the effect of exogenous sfericase protease in commercial broiler diets, with or without protease matrix values included in the feed formulations on growth performance and ileal digestibility of broilers fed low or high amino acids levels. Two experiments (Exp) were conducted. Exp 1 - 1,848 day-old-male chicks Cobb 500 were randomly assigned to 6 dietary treatments with low amino acid (AA) density levels. The experimental design consisted of 2 x 3 factorial with two different formulations: with protease matrix (PM) values included in the formulation – positive control (PC), or without the inclusion of PM in the formulations – negative control (NC); and 3 levels of protease inclusion: 0, 10,000, and 30,000 New Feed Protease (NFP)/kg feed. Exp 2 - 2,100 day old-male chicks Cobb 500 were randomly allocated to 6 dietary treatments, with 14 replicates in 84 floor pens with the same treatment combinations of the Exp1, but with high AA density levels. The difference between low and high AA levels were around 8% (ranging from 1 to 16% depending on the AA and phase). Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were evaluated 7, 21, 35 and 42 d of age. At 42 d and 21 d for Exp1 and Exp2, respectively, 5 birds each pen were used for ileal digesta collection to determine ileal digestibility of dry matter (IDM), crude protein (IDCP) and energy (IDE). Data were submitted to ANOVA using SAS program. Means were compared with Tukey-Kramer test when significant different using at $P < 0.05$. There was no interaction effect among PM formulation and protease levels on performance variables in Exp1 and Exp2 ($P > 0.05$). Also, cumulative FI did not differ in both experiments ($P > 0.05$). In Exp1, broilers fed NC formulation had lower FCR between 1 to 35 d of age compared to birds fed in the PC in Exp1 ($P < 0.05$) and protease inclusion at 10,000 and 30,000 NFP/kg had lower FCR compared to no protease included ($P < 0.05$). No significant difference was found with protease inclusion in FI, BWG, and FCR from all cumulative periods (1 to 21 d, 1 to 35 d, and 1 to 42 d; $P > 0.05$) with high AA levels (Exp2). IDCP was significantly higher in broiler fed NC when compared to broilers fed PC in Exp1 ($P < 0.05$). In Exp1, protease inclusion of 10,000 and 30,000 NFP/kg had higher IDM and IDCP ($P < 0.05$) compared to no protease inclusion, and the protease inclusion of 30,000 had higher IDE compared to broilers fed no protease ($P < 0.05$). In

Exp2, no significant digestibility effects were observed ($P > 0.05$). In conclusion, broilers fed sfericase protease had decreased FCR and increased ileal digestibility, more evidently in diets containing lower AA levels.

Key Words: Performance; Digestibility; Nitrogen; Protease; Sfericase

198 A novel exogenous sfericase protease improved growth performance of broilers fed corn-soy diet. Catarina Stefanello¹, Yuri Katagiri Dalmoro¹, Daniele Pozzebon da Rosa¹, Levy Teixeira², Jose Otavio Sorbara^{2*}, Aaron Cowieson², Murtala Umar Faruk², ¹*Federal University of Santa Maria, Santa Maria, Brazil*, ²*DSM Nutritional Products, Kaiseraugst, Switzerland*.

Two experiments (Exp.) were conducted to evaluate the effects of a novel exogenous sfericase protease on growth performance and digestibility of dry matter and nitrogen of broilers chickens until day 35 of age. In Exp. 1, 1,350 one-day-old male chicks (Cobb 500) were allocated into 54 floor pens and fed a positive control (PC; commercially relevant ME and balanced amino acids); negative control (NC; with reductions of 6% dig. Lys and proportional reductions for adjacent amino acids compared to the PC), or NC supplemented with the novel sfericase at 30,000 NFP (new feed protease units)/kg. The dietary treatments had 18 replicates of 25 birds each, distributed in a completely randomized design. On day 35, ileal digesta was collected to determine the apparent ileal digestibility of dry matter and nitrogen. In Exp. 2, 1,620 one-day-old male chicks (Cobb 500) were allocated in 54-floor pens and fed a control basal diet (Control); Control supplemented with sfericase at 30,000 NFP/kg, or at 60,000 NFP/kg. Birds were distributed in a completely randomized design, having 18 replicates of 30 birds each from day 1 to 35. Data were submitted to one-way analysis of variance using the MIXED procedure of SAS. Means were compared by the Tukey test when the effects of dietary treatments were significant at 5%. In Exp. 2, linear regression equations were fit to the increased levels of the novel sfericase. In Exp. 1, from day 1 to 35, BW gain and FCR of broilers improved 3.4 and 2.5% when diets were supplemented with a novel sfericase, respectively, whereas the digestibility of N increased by 2.7% compared to the NC. In Exp. 2, diets with usual protein and amino acid (AA) levels and supplemented with 30,000 NFP/kg had 2.3 and 1.75% improved BW gain and FCR from day 1 to 35, respectively. When diets were supplemented with 60,000 NFP/kg, BW gain and FCR were enhanced by 3.9 and 3.2%, respectively compared to the Control. In conclusion, a novel sfericase protease resulted in improved body weight gain, feed conversion ratio, and N digestibility in corn-soy diets formulated with or without reduction of protein and digestible amino acids. This provides an opportunity for feed cost optimization all in maintaining broiler performance.

Key Words: Performance; Bacillus; Nitrogen; Protease; Sfericase

199 Protein hydrolysis by a novel sfericase protease combined with endogenous enzymes evaluated in vitro.

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Opposed to common feed enzymes such as phytases or carbohydrases, broilers have their own system of endogenous proteases to digest dietary proteins. Endogenous proteases are present along the gastrointestinal tracts and include different enzymes, such as the acid protease pepsin and pancreatic proteases trypsin and chymotrypsin. Despite good efficiency of this system, some limitations are observed, such as in the presence of trypsin inhibitors or complex proteins, leading to lower extent of protein hydrolysis. To overcome such limitations, exogenous proteases can be supplemented to the broilers diet due to their ability to cleave almost any peptide linkage within protein chains and therefore leading to more extensive hydrolysis. The main objective of this work was to demonstrate how an exogenous protease, a novel subtilisin sfericase protease, improves protein degradation together with endogenous proteases using an *in-vitro* set-up. The *in-vitro* experiment simulated broiler gastrointestinal tract, particularly the crop (pH 5.5, 30 minutes), the gizzard (pH 2.5, 15 minutes) and the small intestines (pH 6.5, 2 hours) steps. Endogenous protease pepsin (3000 U/g diet) was added in the gizzard step, pancreatin (4 mg/g diet) in the intestinal step, while the sfericase protease (30 mg/g diet) was added in the crop phase. Additionally, a control treatment under similar conditions but without the exogenous protease was tested. Samples were collected after each step at 0, 30, 45 and 165 minutes, and supernatants submitted to further analysis. The extent of protein degradation for each timepoint was evaluated using a spectrophotometric assay in which the alpha-amino groups released by hydrolysis react with o-phthaldialdehyde to form an adduct that absorbs at 340 nm; results were analyzed by using One-way ANOVA test. Samples were also evaluated by proteomics method which identifies the specific peptides the different enzymes can hydrolyze. The inclusion of the sfericase protease led to a statistically significant increase in the release of alpha-amino groups (11.6%) after crop step of the *in vitro* simulation, and a significant increase was maintained after the 2 hours intestinal step (7.2%), compared to the control treatment without the exogenous enzyme. Proteomics analysis demonstrated that the addition of the sfericase protease on top of trypsin or pancreatin significantly changed the peptide profile, notably by increasing the degradation of lectin and beta-conglycinin. The results demonstrate that this novel sfericase protease is a valuable additive degrading protein components that endogenous proteases cannot hydrolyze, therefore enhancing protein digestibility.

Key Words: Enzyme; Protease; Exogenous; Proteomics; Hydrolysis

200 Effects of crude protein levels and protease on growth performance, energy utilization, and protein digestibility of broiler chickens. Lina Peñuela-Sierra^{1*}, Paula Lozano-Cruz¹, Jhon Mejia- Abaunza¹, V.L Aragao-Neto¹, Muhammad Ali¹, Joaquin Cabanas-Ojeda¹, Maria Alfaro-Wisaquillo¹, Gustavo Quintana-Ospina¹, Bindhu Vasanthakumari², Alexandra Wealleans², Edgar Oviedo-Rondón¹, ¹*North Carolina State University, Raleigh, North Carolina, United States*, ²*Kemin Industries, Des Moines, Iowa, United States*.

Low crude protein (CP) diets can reduce nitrogen (N) excretion and costs by increasing N utilization efficiency. Exogenous proteases may further improve protein digestibility in low CP diets, but the addition of crystalline amino acids (AA) may cloud this effect. This experiment evaluated the live performance, digestibility, and energy utilization of broilers fed grower diets (8-24 days age) containing three CP levels, with and without protease. A total of 294 Ross 308AP male broilers were placed in 42 battery cages. All chickens were fed one common starter diet in crumbles up to d 7. Six treatments resulted from a 3x2 factorial arrangement of three CP levels (17, 19, and 21% CP) and two protease levels (0 and 300 g/ton), with seven replicates per treatment and seven chicks per cage. Data were analyzed in a completely randomized design. Three isocaloric (3,100 kcal/kg ME) basal diets were formulated to reduce CP, but all diets maintained digestible Lys in 5.47% of CP and the same ideal protein profile. Crystalline Lys, Met, and Thr were used to obtain desired AA levels of balanced protein but not to keep similar AA concentrations across the three diets. The Lys:calorie ratios were 0.30, 0.34, and 0.37 %/Mcal ME. All diets contained titanium oxide as an indigestible marker. The exogenous protease or sand was added on top of these basal diets. Excreta was collected on days 23 and 24. The AME and AMEn, CP digestibility, calorie, and N conversion ratios were determined. Interaction effects ($P>0.05$) were observed on FCR, energy, and CP digestibility. The main effects of CP level were observed ($P<0.001$) in BW at 24 d and BW gain (8-24d). As expected, BW at 24 d (1,405, 1,338, and 1,246 g), BW gain, and FCR (8-24d) worsened as dietary CP reduced from 21 to 17%, and protease did not improve ($P>0.05$) in these parameters. The BW gain decreased by 12.9%, and N intake reduced from 48.96 to 38.49 g/bird. Feed intake was not affected ($P>0.05$) by treatments. The AME and AMEn were better ($P<0.05$) in chickens fed diets without protease containing 17 and 19% CP than those with 21%. Protease supplementation improved ($P<0.01$) AMEn in 71 kcal/kg, CP digestibility from 59.45 to 63.51 %, and ileal DM digestibility from 67.08 to 73.49%, but only in diets containing 21% CP. However, the calorie conversion worsened from 3.66 to 4.25 kcal/g BWG as CP level decreased while N conversion improved from 40.0 to 36.1 g/kg BWG. In conclusion, low

CP diets with balanced protein reduced live performance and improved N utilization but had a detrimental effect on the efficiency of energy utilization. The exogenous protease only improved AME, AMEn, protein, and DM digestibility in diets with 21% CP but did not improve live performance.

Key Words: AMEn; broilers; calorie conversion; exogenous protease; low crude protein

201 Efficacy of a novel microbial serine sfericase endopeptidase on growth performance of broiler chickens fed a wheat-soybean mealbased diet. Farina Khattak², Guenter Pappenberger¹, Jose Otavio Sorbara¹, A. Smith¹, Aaron Cowieson^{1*}, Murtala Umar Faruk¹, ¹*DSM Nutritional Products, Kaiseraugst, Switzerland*, ²*SRUC, Edinburgh, United Kingdom*.

This study investigated the efficacy of a novel microbial serine sfericase endopeptidase protease expressed in *Bacillus licheniformis*, in broilers fed wheat and soybean meal-based diets under simulated commercial conditions. A total of 576, male, day-old Ross 308 broilers were used in this 35-day study in a randomized complete block design. The study contained 2 treatments, each containing 24 replicates. The control cohort was fed a basal diet meeting Ross 308 nutrient requirements. This diet was then supplemented with 30,000 NFP/kg feed protease and represented the second treatment. The experimental diets were fed as crumbs during starter (0-14d) and pellets during grower (14-28d) and finisher (28-35d) phases. Feed and water were supplied ad libitum. Average daily feed intakes (ADFI) and average body weights (ABW) were recorded per pen at 0, 14, 28 and 35-d and average daily weight gain (ADWG) and mortalityadjusted feed conversion ratios (FCRc) were calculated. Data were analyzed by one-way ANOVA with blocks using Genstat 19. The in-feed recovery of protease in starter, grower and finisher feeds were 27,740, 30,460 and 29,520 NFP/kg, respectively. The ABW of birds fed diets supplemented with protease were +17%, +13%, and +11.9% higher ($P<0.05$) during starter, grower, and finisher phases, respectively. The ADFI was also higher ($P<0.05$) during starter (+2.5%), grower (+5.6%), and finisher phase (+7.1%) in birds fed diets containing protease compared with the control. This improvement in ABW and ADFI in protease fed birds was reflected in significantly lower FCR values during starter (-12%) and grower (-5.2%) phase only. Over the entire trial duration (0-35d), birds receiving the diet that was supplemented with protease had +6.3% and +3.3% higher ($P<0.05$) ADWG and ADFI, respectively, and -5.1% lower ($P<0.05$) FCRc values compared to un-supplemented control birds. The overall mortality remained low (3.2%). It can be concluded that this novel sfericase protease is effective in improving broiler growth performance and can be used in animal production industry to optimize broiler performance.

Key Words: Performance; Broiler; Bacillus; Protease; Sfericase

202 A test of concept using alternative ingredients to total replace soybean meal and oil in fast growing male broilers from day 1. Leon Marchal^{1*2} Abiodun Bello³, Eric Sobotik⁴, Gregory Archer⁴, Yueming Dersjant-Li¹, ¹IFF, Oostgeest, Netherlands, ²Wageningen University & Research, Wageningen, Netherlands, ³Danisco Animal Nutrition - IFF, Wilmington, United States, ⁴Texas A&M University, College Station, United States.

Depending on local availability and prices of raw materials reduction of soybean meal (SBM) in broiler diets is of interest to reduce costs. Also, for some geographical regions, to reduce carbon footprint there is growing attention not to import feed raw materials over long distances or from endangered ecosystems. As a most sensitive model fast growing Ross 308 males were used in a full soy free diet from day 1. Raw materials used in the alternative diets were a mix of rapeseed-, sunflower-, cottonseed- and maize gluten meal, pea flour and potato protein concentrate and the synthetic sources of the first 8 limiting amino acids. In total 2574 birds were used for 9 treatments with 13 replicates and 22 birds per pen. A commercially representative wheat, corn, SBM with low inclusion of rapeseed and sunflower meal based diet with phytase (a novel consensus bacterial 6-phytase variant, PhyG) and 750 U/kg xylanase was used as positive control. Two alternative diets were formulated with 1), the same nutritional content as of PC (ALT1); 2) with reduced CP (and higher synthetic amino-acid ratio's) by 2% vs PC (ALT2). All diets were supplemented with 2,000, 1,500 and 1,000 FTU/kg phytase (a novel consensus bacterial 6-phytase variant, PhyG) in starter (0-10d), grower (11-21d)

and finisher 1 and 2 (22-35 and 36-42d) phases. To the alternative diets also 800 U/kg protease was added in all phases. The alternative diet was additionally supplemented with a), the same xylanase level as PC (ALT1+X; ALT2+X); b), mixed enzyme with xylanase at 1200 U/kg and b-glucanase at 152 U/kg (ALT1+XB; ALT2+XB); c), As diets 'b' but supplemented with betaine at 0.8% (ALT1+XB+Bet; ALT2+XB+Bet), d), as diets 'b' but supplemented with probiotic (at 150,000 CFU/g; ALT1+XB+Pro; ALT2+XB+Pro). Data were analyzed as one-way ANOVA. Reducing CP in ALT2 diets improved BW and FCR vs normal CP ALT1 diets, in each dietary group. With low CP diets, ALT2+X reduced final BW from 3256g in PC to 2930g, however, ALT2+XB, ALT2+XB+Bet, ALT2+XB+Pro stepwise improved BW vs ALT2+X (by 38, 42 and 152g respectively). Feed conversion ratio showed similar response as BW. Among the SBM free treatments, ALT2+XB+Pro treatment resulted in the highest final BW (3082g at 42 days, close to the breeder's performance objective at 3136g), although it did not fully recover the performance to the level of PC. However, the feed costs per kg of body weight gain were not statistically significant different between these 2 treatments. It is shown that a reduced crude protein (higher synthetic amino acid ratio) approach combined with a mix of hydrolytic enzymes and consortia of probiotics can be an effective strategy in using less digestible alternative feed ingredients.

Key Words: broiler; enzymes; sustainability; soy free; alternative ingredients

Genetics and Molecular Biology

203 A multi-tissue atlas of regulatory elements in the chicken genome. Zhangyuan Pan^{1*}, Ying Wang¹, Mingshan Wang², Xiaoning Zhu³, Shenwen Gu¹, Conghao Zhong⁴, Liqi An¹, Joana Damas⁵, Dailu Guan¹, Ye Bi¹, Shang Wu¹, Mary E. Delany¹, Hans H. Cheng⁶, Yuzhe Wang³, Congjiao Sun⁴, ¹University of California, Davis, Davis, California, United States, ²State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China, ³State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing, China, ⁴National Engineering Laboratory for Animal Breeding, China Agricultural University, Beijing, China, ⁵The Genome Center, University of California, Davis, California, United States, ⁶USDA-ARS, Avian Disease and Oncology Laboratory, East Lansing, Michigan, United States, ⁷MRC Human Genetics Unit at the Institute of Genetics and Molecular Medicine, The University of Edinburgh, Edinburgh, United Kingdom.

Chicken is one of the most important farm animals in the world. The functional annotation of chicken genome, especially regulatory elements will significantly enhance genom-based biology in fundamental and applied research and improve our genome to phenome prediction. In this study, we systematically identified and annotated the regulatory elements in the chicken genome by integrating 375 genome-wide sequencing datasets including Chromatin Immunoprecipitation Sequencing (ChIP-seq) for 4 histone marks and CTCF, Transposase-Accessible Chromatin (ATAC-seq), Reduced Representation Bisulfite sequencing (RRBS), RNA-seq and Hi-C datasets across 23 tissues in chickens. A total of 15 chromatin states, 1.57 million regulatory elements, and 1.2 million enhancer-gene pairs were identified. We also identified 790,279 tissue-specific regulatory elements and 7,662 super-enhancers. Interestingly, our results revealed that distinct tissue-specific strong enhancers were significantly involved in chicken domestication and genetic selection, such as brain-specific enhancers targeting on *NELL1* under strong selection in domestication, while muscle-specific enhancers targeting on *TEAD4* under strong selection in meat production. The genome-wide association study (GWAS) enrichment results showed the intestine-common regulators were significantly enriched on growth traits. To our knowledge, this is the most comprehensive annotation of regulatory elements in avian genome to date, and this will be a great resource for the animal community to further explore the evolution and causative variants of complex traits in birds.

Key Words: domestication ; regulatory elements; functional annotation; chicken genome; complex traits

204 High environmental temperature regulates hypothalamic autophagy machinery and its upstream regulators in four chicken genetic lines. Craig Maynard^{1*},

Elizabeth Greene¹, Sami Dridi¹, ¹University of Arkansas, Fayetteville, Arkansas, United States.

Autophagy is the cellular process by which the body cleans out damaged or nonfunctioning organelle or cells in order to maintain cellular homeostasis. In addition, autophagy has been identified as a means to conserve cellular resources during starvation by harvesting the aforementioned damaged or nonfunctioning cells to allow for maintenance of healthy cells. Heat stress is one of the major concerns facing the broiler industry and is known to cause a reduction in feed intake among afflicted broilers. Therefore, a study was conducted to determine the effect of heat stress on broiler autophagy machinery and the upstream energy sensor AMPK, and whether these responses vary in three broiler strains [Athens Canadian Random Bred (ACRB); 1995 Random Bred (95RB); 2015 Random Bred (15RB)] and their common ancestor [jungle fowl (JF)]. Results from gene expression analysis showed a significant upregulation of AMPK α 1 and ATG16L1 by exposure to heat stress. Significant responses of gene expression by genetic line showed an upregulation of AMPK β 1 in 95RB broilers compared to 15RB broilers, while JF and ACRB broilers were intermediate. ATG16L1 gene expression was downregulated in ACRB, 95RB, and 15RB broilers compared to JF. These results indicate that high environmental condition and broiler genetic line modulate the hypothalamic expression of autophagy machinery and AMPK, and further in depth investigation into protein expression and other autophagy-related regulators are warranted.

Key Words: autophagy; brain; gene expression; Heat stress

205 Consequences of different shipping methods on the poultry cecal microbiota. Dana Dittoe^{1*}, Maria Rivera¹, Hayley Thompson², Madison Looper², Jordan Looper², Spencer Briggs², Keaton McDorman², Steven Ricke¹, ¹University of Wisconsin- Madison, Madison, Wisconsin, United States, ²University of Arkansas, Fayetteville, Arkansas, United States.

Variations in shipping methods have been shown to generate bias among microbiome results. As such, standard routine shipping practices need to be developed as microbiome sequencing becomes more routine. Therefore, the objective was to determine the impact of different shipping conditions (storage and time) on the microbiota composition of poultry ceca. Broiler ceca were obtained from 12 broilers (42d old) at a commercial processing facility and equally distributed across storage conditions and time (n=12). Storage conditions consisted of 100 mg of ceca being stored in 1.5 mL microcentrifuge tubes at room (20°C, RT), refrigeration (4°C, RF) or freezer (-20°C, FR) temperature, 100 mg of ceca in 95% molecular EtOH at room temperature (EtOH), and 100mg of ceca aseptically

smear on FTA cards and stored at 20°C (FTA). DNA of RT, RF, FR, and EtOH treatments were extracted with a Qiagen Stool Mini Kit and genomic DNA of ceca stored on FTA cards was extracted using a Qiagen DNA Investigator Kit at 0, 48, 96, and 168 h (7d) to represent typical shipping times. Using custom primers, the V4 region of the 16S rRNA gene was sequenced on an Illumina MiSeq. Demultiplexed sequencing data were analyzed using QIIME2 (2022.4) and the microbiota and compositional shifts were determined through taxonomic alignment and the analysis of composition of microbiomes (ANCOM). Taxa across time were primarily represented by genera such as: *Phascolarctobacterium*, *Bacteroides*, *Methanocorpusculum*, *Gastranaerophilales*, *Faecalibacterium*, *Alistipes*, *Clostridia vadinBB60 group*, *Clostridia UCG-014*, *Lachnospiraceae*. At 0h, *Clostridia vadinBB60 group*, *Erwiniaceae*, *Erysipelotrichales*, and *Lachnospiraceae CAG-56* were significantly different when using ANCOM (W=156, 154, 142, 141). At 48h, *Psuedoflavonifractor*, *Lachnospiraceae CHKCI001*, *Metha nomassillicoccus*, *Escherichia-Shigella*, *Lachnospiraceae [Eubacterium] hallii group*, and *Lachnospiraceae CAG-56* were different (W=194, 193, 192, 190, 186, 177). At 96 h of storage, *Lachnospiraceae CHKCI001* and *Lachnospiraceae [Eubacterium] hallii group* were different among the taxa (W=157, 149). At 168 h, *Lachnospiraceae CHKCI001*, *Lachnospiraceae Blautia*, *Lachnospiraceae [Eubacterium] hallii group* and *Butyricicoccaceae UCG-009* were different among taxa at the genus level (W=159, 144, 144, 142). Overall, storage conditions directly impacted the taxa at the genus level with RT and FTA distorting *Lachnospiraceae* populations across time and FR, RF, and EtOH being more stable. Ultimately, these results suggest optimal storage conditions for shipping cecal samples destined for microbiome sequencing are with FR, but shipping under RF or EtOH conditions are also acceptable.

Key Words: ceca; microbiome; poultry; shipping; storage conditions

206 Cytogenetics of lameness; cells that disobey laws of division and make-up their own rules. Paul Cotter^{1*}, B. Fetrow², ¹Cotter Laboratory, Arlington, Massachusetts, United States, ²Maple Leaf Farms, Leesburg, Indiana, United States.

Under normal physiologic conditions (homeostasis) the mitotic rate (mitotic index, **MI**) of avian bone marrow (**BM**) is < 1% and most division occurs among cells of the erythroid series. Inflammation increases the MI, alters the **G:E** (granulocyte: erythrocyte) ratio, and cells of the plasmacyte (**PC**) series are more frequent as a result of invasion and replication of residents. When PC reach 2% a “reactive” plasmacytosis (**RP**) exists (**RPBM**). Lame duckling **RPBM** has been described as an appropriate theater for the study of the protean nature of reactive PC. There some Mott cells, PC with a defective secretory

mechanism, are found to be binuclear and trinuclear. Erythroid cells and other leukocytes also divide at higher rates. Amitosis (cell division without chromosomes or use of a spindle apparatus) and cytogenetic abnormalities are also more common. Here the purpose is to describe cytogenetic abnormalities detected in dividing BM cells of lame ducklings. The method is by a light micrographic examination of Wright-Giemsa-stained touch preparation slides from femur BM samples taken between 1 and 4 wk. All samples were obtained from lame ducklings grown on wood floors covered with shavings. The results: a myriad of atypical dividing cells was detected. The presentation will be directed toward establishing rules for division; mitosis, amitosis, and budding. Examples of cells at various stages of each form of division are chosen for illustration. In each case, how a certain rule is broken is the subject. Cases of amitosis in inappropriate cells (polychromatic RBC) are included. Polyploidy of cells of all lineages, polytene chromosomes in a giant Mott PC; chromosomal bridges, vagrant chromosomes, and cells showing misguided mitotic behavior in BM are included. In some atypical cytokinesis the cell neighborhood was populated by cell associated bacteria (**CAB**) and free-swimming types. In conclusion cells of all lineages populating **RPBM** divide by atypical mitosis, amitosis, and by budding. It is assumed that inappropriate division is directly connected to the clinical picture of lameness, and that it results from exposure to toxins of microbial origin.

Key Words: lameness; amitosis; chromosome; cytogenetics; mitosis

207 Potential role of endoplasmic reticulum stress in broiler woody breast myopathy. Elizabeth Greene^{1*}, Sami Dridi¹, ¹University of Arkansas, Fayetteville, Arkansas, United States.

Genetic selection for high growth rate and feed efficiency in broilers has made spectacular progress, but this advancement has not come without negative consequences. Woody breast (WB) myopathy is one of the most challenging problems facing the poultry industry, particularly since its mechanistic cause is currently unknown. Here, using a primary chicken myotube culture model, we show that hypoxia and endoplasmic reticulum (ER) stress are an integral component of the etiology of the myopathy. For *in vivo* studies, breast muscle from 56d-old broilers were subjectively hand scored by a well-trained person for WB on a scale of 0-3, with 0 showing no signs of WB, 1 was mild, 2 was considered moderate, and score 3 being severe WB. Breast muscle from score 0 (normal) and 3 (severe WB) were either snap-frozen in liquid nitrogen and stored at -80°C for RNA and protein analysis, or fixed in 4% PFA for histological analyses. Primary myoblasts were isolated from breast muscle of E18 embryos and cultured on collagen-coated culture dishes in a humidified atmosphere at 37°C. Hypoxia was induced by placing the cultures into a gas-tight modular hypoxic chamber (1%

O₂/5% CO₂/94% N₂) for either 8 or 24h. The control cells were maintained at normoxic conditions (5% CO₂/95% O₂). Gene and protein expression were determined by real-time qPCR and Western blot, respectively. Data were analyzed by Student's *T*-test or one-way ANOVA, as appropriate. Multiple components of the ER stress response (78-kDa glucose-regulated protein: GRP78; protein kinase R-like endoplasmic reticulum kinase: PERK; and C/EBP homologous protein: CHOP) were significantly upregulated in WB as compared to normal muscle, and this response was mimicked by hypoxic conditions in myotube culture. Additionally, apoptotic pathways, as indicated by increases in caspase 3 gene expression and protein levels and decreases in B-cell lymphoma 2 (Bcl-2) and survivin, were

activated in both WB tissue and myotube culture exposed to hypoxia. Finally, as a phenotypic hallmark of WB is enhanced fibrosis and increased collagen content, we show that hypoxic conditions increase collagen 1A1 and 1A2 gene expression, as well as collagen 1 protein in primary myotubes. These findings indicate that hypoxia and ER stress are present in WB, hypoxia can upregulate the cell death arm of the unfolded protein response (UPR) and lead to collagen production in a culture model of WB, indicating potential mechanistic targets for future interventions to mitigate this myopathy.

Key Words: hypoxia; Woody breast; endoplasmic reticulum stress

Immunology, Health and Disease I

208 Inclusion of a porcine intestinal mucosa hydrolysate (Palbio 62 SP) in broiler chickens during a necrotic enteritis challenge. Matthew Jones^{1*}, Charles Hofacre¹, Sergi Segarra², ¹*Southern Poultry Research Group, Inc., Watkinsville, Georgia, United States*, ²*R&D Bioiberica SAU, Esplugues de Llobregat, Barcelona, Spain*.

Necrotic enteritis is a disease in broiler chickens caused predominantly by cycling of *Eimeria* species coinciding with proliferation of a toxigenic strain of *Clostridium perfringens* (CP). A variety of strategies, including nutritional products, have been used to help control aspects of this disease. Palbio 62 (P62) is a porcine intestinal mucosa protein hydrolysate. While there is data supporting the benefits of P62 in poultry and other animal species, there is little data on efficacy during enteric challenges in broiler chickens. A necrotic enteritis challenge floor pen was conducted to evaluate product efficacy. Birds were allocated into five study groups: a non-CP challenged group (Ctrl), a CP challenge control group (CPCtrl), a challenged group with an ionophore (narasin) /chemical (nicarbazin) product (CPIC), a challenged group with IC plus 1% P62 (ICP1), and a challenged group with IC plus 2% P62 (ICP2). Each group was represented by 12 replicate pens of 25 male Ross 708 broiler chickens. All chicks were vaccinated with one dose of commercial coccidia vaccine on day of placement per manufacturer guidelines. A toxigenic strain of CP was administered to each pen on day 15, 16, and 17. On day 18, NE lesions were evaluated, and excreta was collected to evaluate OPGs. Birds and feed were weighed on days 0, 15, 35, and 42 for performance data. Data were subjected to an ANOVA and means were compared using an LSD test with a p-value of 0.05. The CPCtrl group had 10.67% necrotic enteritis mortality indicating a successful challenge. All other groups had statistically lower NE mortality. Oocyst counts were lower in all groups receiving IC. From 0-15 days as the coccidia vaccine was cycling and ramping up in numbers, ICP1 had greater weight gain and lower feed conversion. By 35 days, birds had time to recover from the CP portion of the challenge. While CPIC had greater body weight gain (2.012 kg) than CPCtrl and Ctrl (1.932 and 1.959 kg, respectively), both ICP1 and ICP2 had further improvement (2.150 and 2.063 kg, respectively) compared to CPIC. Feed conversion was also improved in ICP1 (1.46) and ICP2 (1.47) relative to CPIC (1.52). By 42 days, there was a maintained advantage in body weight gain in ICP1 relative to CPIC. The CPIC group had lower feed conversion than Ctrl and CPCtrl. Feed conversion was further reduced in the P62 groups at 42 days. Early ability to improve body weight and feed conversion demonstrated P62's efficacy during the stress created by the cycling protozoa. This porcine intestinal mucosa protein hydrolysate improved performance metrics out to processing ages. This study indicates that P62 could be used

in non-vegetable-based diets to support recovery from necrotic enteritis.

Key Words: *Clostridium perfringens*; enteric health; nutrition; *Eimeria*

209 Calprotectin as a fecal biomarker for Necrotic enteritis in chickens. Mahmoud Mahmoud^{1*}, Craig Baumrucker¹, Vinicius Buiatte¹, Dorian Dominguez¹, Celia O'Brien², Mark Jenkins², Alberto Gino Lorenzoni¹, ¹*Pennsylvania State University, University Park, Pennsylvania, United States*, ²*Agricultural Research Service, Department of Agriculture, Beltsville, Maryland, United States*.

The reduced use of antibiotics as growth promoters in poultry has increased the incidence of clinical and subclinical necrotic enteritis (NE) in chickens. Necrotic enteritis is a serious intestinal disease caused by *Clostridium perfringens*. This disease is linked to mortality, reductions in performance, and decreased uniformity within the flock. A careful necropsy conducted by specialized professionals is needed to diagnose NE in poultry, which is a time-consuming and expensive process. A simple, non-invasive method for the diagnosis of necrotic enteritis is needed for commercial poultry and poultry-related research. Calprotectin is a protein present in cells of the innate immune system. Elevation of calprotectin in the fecal matter has been associated with chronic IBD (inflammatory bowel disease) in humans. The present study aimed to detect the presence of calprotectin in the fecal samples of chickens undergoing necrotic enteritis. Four hundred Ross 308 broilers were allocated into control and infected treatments. Both treatments received diets containing wheat and fishmeal as predisposing factors for necrotic enteritis. Birds in the infected treatment were challenged with 5,000 oocysts of *Eimeria maxima* on days 13 and 1 mL of 1 x CFU of *Clostridium perfringens* per bird on days 18 and 19. On day 21, five birds from each pen were randomly selected and euthanized. A necropsy was performed for collecting fecal samples and lesion scoring. Sixteen fecal samples were selected from birds without lesions (n=4 birds), birds with moderate lesions (n=4 birds), and birds with severe lesions (n=8 birds). The NP-40 lysis buffer was used to extract the protein from the samples. The samples were analyzed using a sandwich ELISA (CHF100094) according to the instructions of the manufacturer. The GraphPad Prism 9 software was used to calculate the protein concentration in the fecal samples. Fecal calprotectin was detected in seven out of the eight samples of birds with severe intestinal lesions (average concentration: 72.73 ng/g). There was no calprotectin detected in the birds with moderate intestinal lesions. Calprotectin was present in one sample from the control samples (average concentration: 24.90 ng/g).

Key Words: *Clostridium perfringens*; Necrotic enteritis; Chicken; Biomarker; Calprotectin

210 Recombinant live therapeutic for prevention and control of necrotic enteritis associated with *Clostridia perfringens* in poultry. Yiannis Kaznessis^{1*}, Kathryn Kruziki¹, ¹*General Probiotics, Inc., St. Paul, Minnesota, United States.*

The objective of this talk is to present the development and testing of a recombinant antimicrobial live therapeutic for prevention and control of necrotic enteritis associated with *Clostridia perfringens* in broiler chickens. The mode of action involves the live therapeutic being administered orally in chickens and reaching the upper GI tract of birds. The recombinant microbe is engineered to express and secrete enterocin A, which exhibits potent inhibition of *Clostridium perfringens*. In the US, a recombinant live therapeutic is considered an animal drug. In 2020, we opened an Investigational New Animal Drug file with the Center for Veterinary Medicine. In the context of the approval path, we tested recombinant live therapeutics in an animal trial and demonstrated the efficacy in a necrotic enteritis model. We also collected data related to the risk recombinant live therapeutics pose to the environment and to human food safety. This study was conducted at Blue River Research Services with 112 floor pens and each with the dimension of 4' x 4'. This study employed the randomized block design. The study utilized 2576 commercial male broilers (Ross 708) from a nearby hatchery in NE floor pen challenge model. Each pen was an experimental unit. The study began on Day 0 (arrival) and ended on Day 42. There were eight treatment groups: TG1: negative control without challenge TG2 and TG3: two controls with two different challenge levels of *Clostridia perfringens* TG4 one positive control (BMD) with high level challenge TG6, TG7, TG8, TG9 two test article groups (GP1 and GP2 are auxotroph variant live therapeutics) with two different challenge levels. Each treatment group used 14 pens, i.e. replicates. Each pen housed 23 birds on day 0. For every endpoint subjected to statistical analysis, a 2-sided test of non-zero difference was conducted at the 0.05 significance level for pairwise group comparisons. No multiplicity adjustment was applied. Statistical analysis was performed using SAS version 9.4 (SAS Institute, Cary, NC). We compiled data from this study and communicated to FDA the following: Mortality was significantly decreased ($p < 0.05$) in treated groups during the necrotic enteritis phase. There was no statistically significant difference in mortality between BMD-treated, GP1-treated and GP2-treated birds ($p < 0.05$). Less than 1% of the organisms administered to chickens can be found overall in the environment on day 42. Results will be presented that were communicated to the FDA to support the assessment that live therapeutics pose a low risk to the environment and to human food safety. The FDA concurred with this

assessment and granted a categorical exclusion claim for the investigational use of live therapeutics.

Key Words: antimicrobial; live therapeutic; animal drug

211 Subclinical doses of combined fumonisins and deoxynivalenol predispose *Clostridium perfringens*-inoculated broilers to necrotic enteritis. Revathi Shanmugasundaram^{1*}, Daniel Adams³, Shawn Cunningham¹, Todd Applegate³, Anthony Pokoo-Aikins⁴, Shelby Ramirez², G. Raj Murugesan², Drew Olson¹, Mitchell Trevor¹, Anthony Glenn¹, ¹*USDA, Athens, Georgia, United States*, ²*DSM Animal Nutrition and Health, Wurmisweg 576, 4303 Kaiseraugst, Switzerland*, ³*University of Georgia, Athens, Georgia, United States*, ⁴*USDA ARS, Watkinsville GA, Georgia, United States.*

Fumonisin (FB) and deoxynivalenol (DON) are mycotoxins that frequently contaminate feedstuffs and cause gut integrity loss, which may predispose broiler chickens to necrotic enteritis (NE). The objective of this study is to identify the effects of subclinical doses of combined FB and DON on gut permeability, necrotic enteritis severity, immune parameters, and overall performance of birds induced with NE. A total of 480 day-old male broiler chicks were divided into four treatment groups; 1. Control group (Basal diet + *Clostridium perfringens*); 2. Necrotic enteritis group (Basal diet + *E. maxima* + *C. perfringens*); 3. FB+DON group (Basal diet + 3 mg/Kg FB + 4 mg/Kg DON + *C. perfringens*); and 4. FB+DON+NE group (Basal diet + 3 mg/Kg FB + 4 mg/Kg DON + *E. maxima* + *C. perfringens*). Birds in NE and FB+DON+NE groups received 2.5 X 10³ *E. maxima* on d 14. All birds were inoculated with *C. perfringens* on d 19, d 20, and d 21. On d 14 (pre-challenge), birds in the FB+DON treatment had lower ($P < 0.05$) body weight gain (BWG) and 21 points ($P = 0.05$) increase in feed conversion ratio (FCR) compared to the control group. On d 35, birds in the NE, FB+DON, and FB+DON+NE groups had 242, 84, and 339g lower BWG and 19, 2, and 22 points increase in FCR than those in the control group. Subclinical dose of FB+DON increased ($P < 0.05$) the NE lesion scores compared to that in the control group on d 21. The presence of FB+DON in NE-induced birds further increased ($P < 0.05$) the NE lesion scores compared to that in the NE group. On d 21, birds in the NE, FB+DON, and FB+DON+NE groups had a 150, 51, and 293% increase ($P < 0.05$) in the serum FITC-D, respectively, and lower ($P < 0.05$) jejunal claudin-1, claudin-2, and zona occludens-1 mRNA levels, and a 4-fold increase ($P < 0.05$) in cecal tonsil IL-1 mRNA level compared to that in the control group. On d 21, birds in the NE group had a 24% decrease ($P < 0.05$) in villi height to crypt depth ratio compared to the control group and the presence of FB+DON in NE-induced birds further decreased the villi height to crypt depth ratio by 8.4% scores compared to that in the NE group. On d 21, d 28, and d 35, birds in the NE, FB+DON, and FB+DON+NE groups had an approximately 1.3-fold increase ($P < 0.05$) in *C.*

perfringens loads in the cecal tonsils compared to that in the control group. On d 21, birds in the FUM+DON group had a lower ($P < 0.05$) Lactobacillus load in the cecal content and a lower ($P < 0.05$) CD8+: CD4+ cell ratio in the cecal tonsils compared to those in the control group. It can be concluded that subclinical doses of combined FB and DON predisposes *C. perfringens*-inoculated birds to NE, and the presence of FB+DON in NE-induced birds exacerbated the severity of NE.

Key Words: Necrotic Enteritis; Gut health; Deoxynivalenol; Fumonisin; Mycotoxin

212 Thyme essential oil and its phenolic derivatives are effective anticoccidial and antibacterial against *Eimeria tenella* and *Clostridium perfringens* in vitro. Martina Felici^{1*}, Benedetta Tugnoli², Giulia Giovagnoni¹, Andrea Piva¹, ²Ester Grilli¹, ³University of Bologna, Ozzano dell'Emilia, Italy, ²Vetagro S.p.A., Reggio Emilia, Italy, ³Vetagro Inc, Chicago, Illinois, United States.

Coccidiosis, caused by protozoan parasites of the genus *Eimeria*, is one of the main threats for aviculture, as it causes severe hemorrhagic diarrhea and acts as a predisposing factor for necrotic enteritis, whose causative agent is *Clostridium perfringens*. The aim of this study was to investigate *in vitro* the anticoccidial and anticlostridial efficacy of thyme essential oil and its main phenolic bioactive compounds (thymol, carvacrol and eugenol) against *E. tenella* and *C. perfringens*, respectively by invasion and minimal inhibitory concentration (MIC) assays, in comparison with conventional antibiotics. For the invasion assay, confluent Madin-Darby bovine kidney cells were challenged with *E. tenella* sporozoites ($5 \times 10^4/\text{cm}^2$), without (control) or with various treatments: salinomycin (5 ppm), thyme oil (40 ppm, thymol content 50%), thymol, carvacrol or eugenol (20 ppm each). After 2 and 24 h at 37°C the efficiency of invasion was determined by counting free sporozoites left in the medium outside cells. For the MIC assay, a broth microdilution method on 96-well microtiter plates was used to test a range of concentrations (2-fold dilutions) of bacitracin (64-0.5 ppm), thyme essential oil (2000-62.5 ppm, thymol content 50%), thymol, carvacrol or eugenol (7.5-0.23 mM). *Clostridium perfringens* ATCC® 13124 strain (10^5 CFU/mL) was incubated with the substances under anaerobic conditions in Reinforced Clostridial Medium at 37°C for 24 h. After incubation, the absorbance at 630 nm was read with a spectrophotometer to measure bacterial growth. The MIC was defined as the lowest concentration that resulted in null absorbance. Data were analyzed with one-way ANOVA and differences considered significant at $p < 0.05$. The invasion assay showed that all the treatments significantly inhibited *E. tenella* invasion efficiency compared to the control already after 2 hours by 55% on average and similarly at 24 h with the highest reduction for salinomycin (-54% compared to control) and an average reduction of 35% for all the botanical treatments ($p < 0.001$).

Against *C. perfringens* the MIC values were found for bacitracin at 2 ppm, for thymol and carvacrol at 1.87 mM, for eugenol at 3.75 mM and for thyme oil at 500 ppm (equivalent to 1.66 mM of thymol). In conclusion, both thyme essential oil and its main phenolic bioactive compounds demonstrated anticoccidial and bactericidal activity against *E. tenella* and *C. perfringens* *in vitro*. Thus, these botanicals could be valuable tools to counteract the causative agents of avian coccidiosis and necrotic enteritis.

Key Words: Clostridium perfringens; feed additives; Eimeria tenella; botanicals; MIC

213 Characterization of probiotic strain G108 for anti-clostridial activity. Lisa Bielke^{1*}, Michael Trombetta¹, Kaylin Chasser¹, Audrey Duff¹, Kate McGovern¹, Dongping Wang², LeAnn Johnston², Hongyu Xue², ¹Ohio State University, Wooster, Ohio, United States, ²Amlan International, Vernon Hills, Illinois, United States.

Necrotic enteritis is an opportunistic disease primarily caused by overgrowth of *Clostridium perfringens* in the small intestine. The objective of these studies was to characterize a novel probiotic strain G108 (Amlan International, Chicago, IL) for effectiveness against *C. perfringens* via *in vitro* and *in vivo* tests. The G108 strain was tested against 9 strains of *C. perfringens* to determine overarching anti-clostridial effect. In agar overlay tests, differences in zones of inhibition ($p < 0.05$) were noted when G108 was compared to non-probiotic bacteria. Next, a germination assay, in which ability of spores to survive pelleting temperatures and grow at the internal body temperature of chickens was completed. A lack of significant change ($p = 0.096$) in cell recovery was indicative of the probiotic's ability to endure feed manufacturing and grow within the gut. A simulation digestive assay was performed to mimic crop, proventriculus, and intestines to ensure the probiotic strain could maintain anti-Clostridial activity within the gut before moving to *in vivo* tests. The assay included 10^8 CFU *C. perfringens* strain TXAM 020410 in the small intestines portion when 10^6 spores of G108 strain were included throughout the entire assay. The reduction in *C. perfringens* had a $p\text{-value} = 0.112$ with a reduction of nearly 1-Log₁₀ CFU. To ensure the *Bacillus* probiotic endured in live birds, single-dose gavage and continued feed application were compared. Every 24 h for 5 d, 10 birds per treatment were euthanized for sampling of crop, ileum, and ceca, plus 12 pooled fecal samples. At all time points and across all sampled locations the group consistently fed the spores had a significantly higher ($p\text{-value} < 0.01$) spore recovery than the gavaged group and more consistent recovery across all locations in the gut. Finally, the G108 strain was included in a necrotic enteritis disease model in which it was added to feed at 10^6 CFU/g and necrotic enteritis induced via pre-dosing with *Eimeria maxima* followed by inoculation with *C. perfringens*. Compared to the diseased control group, inclusion of G108 strain significantly ($p < 0.05$) improved %

change in BWG during the necrotic enteritis period and decreased lesion scores. Also, *C. perfringens* was measured in the ileum and probiotic treated groups had less ($p < 0.05$) CFU/g than disease control. Collectively, the probiotic strain G108 demonstrated broad and effective anti-clostridial properties both *in vitro* and *in vivo*, exhibited resilience in harsh environments, and thus can be used as an effective, natural alternative to in-feed antibiotics for necrotic enteritis control.

Key Words: *Clostridium perfringens*; DFM; necrotic enteritis; *Eimeria*; probiotic

214 The effect of necrotic enteritis challenge on production performance, cecal microbiome, and cecal tonsil transcriptome in broilers. Gabriel Akerele¹, Walid Al Hakeem^{1*}, Jeferson Lourenco¹, Ramesh Selvaraj¹, ¹*University of Georgia, Athens, Georgia, United States.*

Necrotic enteritis (NE) infection induces intestinal lesions, decreases gut permeability, alters the composition of gut microbiota, and genes involved in metabolism. The objective of this study was to identify the effects of experimental NE infection on the production performance, gut microbiome, and cecal tonsil transcriptome in broiler birds. A total of 192 chicks were randomly allocated into two treatments: 1) control and 2) NE challenge. Each group was replicated in eight battery cages with twelve birds per cage. NE challenge was induced by inoculating *Eimeria maxima* at 14 d of age and *Clostridium perfringens* at 19, 20, and 21 d of age. Bodyweight and mortality were recorded on 7 and 14 post-*Eimeria* infection (dpi). NE lesion score, cecal microbiota, and cecal tonsil transcriptome were evaluated at 6 dpi. Cecal and ileal *C. perfringens* loads were measured at 5, 6, and 8 dpi. Gut permeability was measured using FITC-d assay at 5, 6, 7, and 8 dpi. Oocyst shedding was measured at 5, 6, 7, 8, and 14 dpi. Parametric analyses were carried out using the student's t-test. Nonparametric analyses were carried out using Wilcoxon's test. NE challenge increased ($P < 0.01$) NE lesion score at 7 dpi, decreased ($P < 0.01$) average weight gain and increased ($P < 0.01$) mortality at 7 and 14 dpi. NE challenge increased ($P < 0.05$) gut permeability at 5, 6, and 7 dpi and increased ileal *C. perfringens* load at 5 dpi. NE challenge increased ($P < 0.01$) *Eimeria* oocyst shedding at 5, 6, 7, 8, and 14 dpi. NE challenge decreased ($P < 0.05$) the relative abundance of *Lactobacillaceae* and increased ($P < 0.05$) the relative abundance of *Campylobacteraceae*, *Comamonadaceae*, and *Ruminococcaceae* at 6 dpi. NE challenge altered the RNA-seq profile by upregulating ($P < 0.05$) vanin 2, chloride channel accessory 1, and sodium channel epithelial beta 1 genes and downregulating ($P < 0.05$) genes involved in lipid metabolism, namely stearyl-CoA desaturase, and fatty acid desaturase-2 at 6 dpi. It can be concluded that NE infection decreased beneficial bacteria in the ceca, increased gut permeability, decreased average body weight gain, and altered lipid metabolism genes.

Key Words: Broilers; Necrotic enteritis; Microbiome; Transcriptome

215 Effect of a *Bacillus subtilis* strain on performance and gut integrity in broiler co-infected with *Clostridium perfringens* type A producing toxin and *Eimeria* spp. Bruna Belote^{1*}, Wanderley Quinteiro-Filho², Naiara Fagundes², Diogo Taschetto³, Andre Favero³, Liris Kindlein⁴, Sergio Vieira⁴, ¹*ISI Institute, Curitiba, Brazil*, ²*Adisseo, São Paulo, Brazil*, ³*Granja Santa Livia, Garibaldi, Brazil*, ⁴*UFRGS, Porto Alegre, Brazil.*

Bacillus subtilis (BS) is an ideal multifunctional probiotic, with great potential for preventing the growth of pathogenic bacteria and enhancing nutrient assimilation. A trial was conducted to evaluate the effects of a in feed probiotic strain of BS on performance and intestinal integrity by I See Inside (ISI) methodology. A total of 660 one-day-old male Cobb x Cobb 500 were used to evaluate the effects of a in feed probiotic strain of *Bacillus subtilis* on performance and intestinal integrity. Birds were allocated in a commercial farm within 30 experimental floor pens (1.65 x 1.65 m), completely randomized block with 10 replicates of 22 birds each, using reused litter after one crop. Mash diet based on corn-soy all vegetable feeds in a 3-phase feeding program (starter – 0 to 21 d; grower – 22 to 35 d; finisher – 36 to 42 d). Feeds and water were provided *ad libitum*. At d 2 of the trial, all birds were challenged with *Eimeria* live vaccine 10x the manufactured recommendation dose. At d 10, 11 and 12, a pathogenic strain of *Clostridium perfringens* type A from field, in a final concentration of 1×10^8 CFU/ml/bird each dose/day by oral gavage. The treatments were CN: Control group, ENRA: Enramycin 10 ppm, and BS: *B. subtilis* commercial product Alterion®, 1×10^8 UFC/500 g. Shapiro-Wilk normality test was applied, non-parametric data were compared using Kruskal wallis test ($P < 0.05$) and parametric data were compared using ANOVA, followed by Tukey test ($P < 0.05$) using Statistix10 software. The bird's performance was evaluated for feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR). Ileum was sampled for histological analysis using the ISI method at 18 and 26d of age. The ENRA and BS groups showed equal results of FCR at 42d ($P > 0.05$; 1,642 x 1,642, respectively), and better results ($P < 0.05$) when compared with CN group (1,677). No significant difference in FI and BWG were observed. At 18d, no significant difference ($P < 0.05$) was observed between the groups in the sum of all the parameters evaluated (ISI total). However, at 26d despite having high score caused by challenge, the BS group showed better ($P < 0.001$) results in ISI total score (6.21) compared to the ENRA group (8.63) due to low scores ($P < 0.05$) for inflammatory cell inflammation in epithelium and in lamina propria compared to the other groups. The results demonstrated that using BS as a feed additive may improve the gut integrity in birds challenged with *Eimeria* and *C. perfringens*, reflecting in the improvement of FCR.

Key Words: Probiotic; Coccidiosis; Histology; ISI; Clostridiosis

216 Constructing shotgun library for *Clostridium perfringens* vaccine. Ying Fu¹*, Hong Wang¹, Tahrir Alenezi¹, Ayidh Almansour¹, Xiaolun Sun¹, ¹University of Arkansas, Fayetteville, Arkansas, United States.

Necrotic enteritis (NE) poses a serious challenge to poultry industry with largely unknown mechanism. In another study, we found that immunized chickens with *C. perfringens* sporulation protein vaccines reduced chicken NE. Based on that finding, in this study, we aimed to develop recombinant vaccines targeting the sporulation protein antigens. Chickens or mice were immunized with *C. perfringens* sporulation protein (Cp-spor-super). *C. perfringens* genomic and plasmid DNA were isolated by phenol/chloroform and bead beater method. The *C. perfringens* DNA was separated on 1% gel and fragments between 0.3-2 kb were collected. The *C. perfringens* DNA fragments were repaired and phosphorylated and then blunt-end ligated with Inadapt-1: 5'-TGATGA-3' and Inadapt-2, 5'-AGTAGTTCATCA-3'. A phagemid of pComb3xSS digested with SfiI was ligated with Vecadapt-For: 5'-TGATGAAGTACTCCT-3' and Vecadapt-Rev: 5'-TGATGAAGTACTGGC-3'. After gel separation and purification, the *C. perfringens* DNA and ligated pComb3xSS were annealed and transformed into TG1 cells to generate bacterial shotgun library (SGL) of Cp-SGL-pComb3. The bacterial library was then used to generate Cp-SGL-phage library using M13O7 helper phage and kanamycin. Bio-panning of phages with the chicken or mouse sera in a plate was performed to select Cp-SGL-phage expressing proteins binding to the anti-*C. perfringens* antibodies in the sera. As a result, sera from chickens or mice immunized with Cp-spor-super were collected and stored at -80 °C. Notably, gel image showed that *C. perfringens* DNA was fragmented into around 1 and 2.5 kb bands using phenol/chloroform and bead beater method. Gel image of Cp-SGL-pComb3 library PCR showed bands from 0.3 to 1.5 kb. Gel image of Cp-SGL-phage library PCR showed bands around 0.6 to 1.5 kb. After 2 cycles of Bio-panning, gel image of eluted Cp-SGL-phage library PCR showed bands around 0.6 to 1.5 kb. In conclusion, we have generated *C. perfringens* shotgun bacterial and phage library. The following-up is to identify and characterize the individual DNA sequences for potential vaccine candidates.

Key Words: Necrotic enteritis; sporulation; Shotgun library; Phage Display

217 The incredible edible egg: dried egg product improves broiler growth performance and supplements cocci vaccines in *Eimeria* and necrotic enteritis challenged birds. Pradyut Paul¹*, Revathi Shanmugasundaram², Kimberly Livingston¹, ¹Optum

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Eggs are nutritionally complete with easily digestible nutrients and immunomodulating proteins. Coccidiosis is a prevalent disease that plagues the poultry industry. Conventionally it is managed by ionophores, but consumer pressures arising from potential concerns of residue in poultry products necessitate a search for alternatives. One such alternative is Coccidiosis vaccine. Although vaccines provide adequate protection against *Eimeria*, it often comes at the cost of early growth performance and can lead to secondary infections resulting in necrotic enteritis (NE). We performed 2 studies to determine if enhanced dried egg product (eDEP) improves broiler performance after administration of a cocci vaccine at day of hatch (EXP1) and reduce the incidence of secondary infections that lead to NE (EXP2). In EXP1, 4 treatments were utilized to determine if eDEP improves growth performance after a cocci vaccine given at hatch and an *Eimeria maxima* challenge at 10D. This resulted in a 2x2 factorial arrangement with 1. Non-vaccinated, challenged control (CON), 2. Vaccinated, challenged (VAC), 3. eDEP fed to 28D, challenged (eDEP), 4. Vaccinated +eDEP fed to 28D, challenged (V+D) with 16 replicates/treatment and 20 animals/pen reared to 42 days of age on used litter. In EXP2, the same treatments were utilized, but chicks were challenged with *Clostridium perfringens* (CP) at 15, 16, and 17D. There were 10 replicates/treatment and 50 birds/pen. At 17D, 4 birds were euthanized, lesion scores were evaluated, and cecal tonsils were collected to evaluate cytokine mRNA expression. Performance was analyzed as a 2-way ANOVA in SAS®. 14D body weight (BW) and average daily gain (ADG) was improved in eDEP supplemented chicks regardless of VAC ($P=0.01$). No other performance parameters were impacted by the treatments. Final BW ($P<0.001$) and overall feed conversion ratio (FCR) ($P=0.04$) was negatively impacted by VAC in EXP2. Yet, eDEP birds had greater final BW regardless of VAC ($P<0.001$) and improved FCR when provided eDEP along with VAC ($P<0.001$). Lesion scores were more prominent in VAC chicks ($P<0.001$), which eDEP reduced ($P<0.001$). IFN γ and IL-10 expression was upregulated in VAC chicks ($P<0.01$). eDEP brought both cytokines to near normal expression levels. In summary, eDEP counteracts the negative impacts of VAC on broiler performance and reduces susceptibility to NE by promoting inflammatory homeostasis.

Key Words: coccidiosis; necrotic enteritis; vaccine; Dried egg Product

218 Effect of direct fed microbial blend and xylanase (EnzaPro) on cecal colonization and immune response of *Salmonella* challenged broilers. Sudhir Yadav¹*, Yunmei Lin¹, Sandra Rodrigues¹, Rasha Qudsieh¹, ¹BioResource International, Durham, North Carolina, United States.

A study was conducted to evaluate the effect of a unique combination of direct fed microbial and xylanase (EnzaPro) on *Salmonella* Heidelberg colonization and immune response of broiler chickens' when raised up to 28 days. A total of 800, one-day-old Ross 708 male chicks were randomly distributed into 4 dietary treatments with 100 chicks per pen in a challenged and similar non-challenged pens where the individual bird was the experimental unit, and the pens were divided to avoid cross-contamination. Corn-SBM-based mash diets were fed (0-28 d). The dietary treatments were control (C; no additives), antibiotic growth promoter (C+ antibiotic; AGP), EnzaPro (C+ EnzaPro; EP), and EP with 75 kcal/kg reduced metabolizable energy (ME) compared to control (C+ EnzaPro - 75kcal/kg; EP-75). The reason for using EnzaPro with/without energy reduction is that xylanase has an energy matrix of 75kcal/kg as well as tested for "on top" effect of EP against *Salmonella* colonization. On day 3, challenged group pens received oral gavage to 20% of the birds with 10^8 CFU/mL/bird of *Salmonella* Heidelberg (seeders), and the remaining 80% were considered non-seeder. Ceca were collected on days 7, 14, 21, and 28 of age from non-seeder birds, and 14 and 28 d of age for seeder birds in the challenged pens. Non-challenged pens were also sampled (at 14 d and 28 d) to ensure the absence of *Salmonella*. On each sampling day, 10 birds per treatment were sampled. Means were separated using Kruskal Wallis test and declared significant at $P < 0.05$. *Salmonella* was not detected in the non-challenged pens. For the challenged pens, on 14 d, all seeder and non-seeder birds were confirmed infected. By day 28, 50% of the seeder birds recovered in EP birds compared to 40% in the control. For the non-seeder birds, recovery by 28 d was 70% in EP birds compared to 45% in control birds. There were no differences in monocytes and heterophils percentages among treatments on 14 and 28 d. However, T helper cells percentage was higher in EP birds compared to AGP at 14 d, and both control and AGP birds on 28 d. Furthermore, EP birds had a higher percentage of T helper cells targeted for intestinal mucosa on 28 d compared to other treatments. There were no differences among treatments in the C-reactive protein (CRP), naive or activated cytotoxic T cells. Based on these results, EnzaPro may increase the recovery rate in birds, infected due to horizontal transmission of *Salmonella* (80% of the flock) compared to AGP and control. This study also provide insight into potential role of EP to enhance the adaptive immune system via T helper cells which regulates IgA in intestinal mucosa leading to decrease in *Salmonella* colonization in broilers.

Key Words: broiler; immune system; *Salmonella*; probiotic; xylanase

219 Indices of intestinal function, health, integrity, and immunity in broiler chickens as influenced by broiler breeder age and enzymatically treated yeast supplementation in diets fed to younger breeder chicks. Anderson Maina^{1*}, Aizwarya Thanabalan¹, Jessica

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The impacts of broiler breeder age on gut health, function indices, and feedstuffs' role in facilitating immunocompetence in younger breeder chicks (YBC) are unexplored. This study assessed organ weight, gut health, and immunity indices in older breeder chicks (OBC) and YBC and responses to feeding enzymatically treated yeast (HY40) in YBC. Fertile eggs: 2,250 (56.5 ± 3.4 g) of 30-wk-old and 550 (64.2 ± 4.2 g) of 47-wk-old Ross 708 BB were hatched. Based on initial body weight (BW), chicks were placed in floor pens (YBC=40 pens, OBC=8 pens; 44 chicks/pen) for a 42-d trial. Five corn and soybean meal-based treatments were formulated to contain 0, 0.05, 0.10, 0.20, and 0.40%HY40 for a 3-phase feeding program: starter: d 0-10, grower: d 11-24, and finisher; d 25-42 and were allocated within YBC (n=8) in a completely randomized block design; OBC was fed a 0%HY40 diet. Feed and water were given *ad libitum*. Two birds per pen were sampled for plasma, jejunal tissues, and relative weight of organs on d 10 and ceca digesta on d 24. Litter was collected on d 35 and 42 and oven-dried at 60 degrees celcius for 4 days for moisture content. Data were subjected to GLM procedures of SAS with treatment as a fixed factor. LSmeans were separated using Tukey test and curve response for HY40 inclusions. Treatments had no ($P > 0.05$) effect on liver weight. Although both treatments had similar spleen weights, bursa weights were higher in OBC than 0% HY40 YBC. Jejunal villi heights were higher in OBC than 0%HY40; villi heights to crypt depth ratio were higher in OBC than 0%HY40 ($P = 0.01$). Lower sodium-dependent neutral amino acid transporter (BOAT1) was observed in OBC than in 0%HY40, whereas jejunal IgA was higher in OBC than 0%HY40. In YBC, 0.2% HY40 bursa were heavier ($P = 0.01$). Bird spleens were heavier ($P = 0.038$) in HY40 treatments than in 0%HY40. Jejunal villi height increased ($P = 0.01$) with increasing HY40; 0.4%HY40 had the tallest VH ($P = 0.01$), 0.05%HY40 YBC had shorter ($P = 0.01$) VH than $\geq 0.1\%$ HY40. Increasing HY40: VH to CD ratio increased ($P = 0.01$), increasing linearly ($P = 0.002$) and quadratically ($P = 0.006$). HY40 increased superoxide dismutase (SOD) ($P = 0.001$) compared to 0%HY40. Inclusion of HY40 at $\geq 0.05\%$ had higher ($P < 0.01$) BOAT1 than 0%HY40; HY40 increased jejunal and plasma IgA ($P = 0.01$) but had no effects ($P > 0.05$) on total bacteria, short-chain fatty acids concentration in digesta on d 24 and litter moisture ($P > 0.05$). In conclusion, although OBC showed more positive gut health indices than YBC HY40 lots, due to inherent maternal age benefits, HY40 improved the same as YBC. This was attributed to the immunomodulatory effect of enzymatically treated yeast, with jejunal histomorphology and plasma IgA being major indicators.

Key Words: Broiler chickens; Gut integrity; enzymatically treated yeast ; Broiler breeder age; Plasma IgA

220 Algal polysaccharides to improve gut barrier function. Marie Gallissot¹, Maria Garcia¹, Franciele Giacobbo^{1*}, Pi Nyvall-Collen¹, Mustapha Berri², ¹*Olmix Group, Brehan, France*, ²*INRAE, Nouzilly, France*.

Recent research has highlighted the potential of in-feed marine macroalgal polysaccharides to strengthen animal's gut barrier function. The cell wall of marine algae is mainly composed of water-soluble sulfated polysaccharides that present diverse biological activities such as reinforcement of gut barrier components via the induction of mucin secretion *in vivo* in the intestinal mucosa (Barcelo *et al.*, 2000). Previous research has shown, *in vitro*, that a red algal extract from *Solieria chordalis* upregulates the gene expression of tight junction proteins and mucin proteins which determine intestinal integrity (Intestinal Biotech Development, 2017). The ability of the red algal extract from *Solieria chordalis* to reinforce the epithelium barrier function was evaluated in a research project in collaboration with INRAE (2019, unpublished data) using several epithelial cell lines. Firstly, an intestinal cell line (IPEC-1) was cultured in the cell-culture medium alone (negative control), incubated with *E. coli* K88 1305 (1.35×10^8 CFU/mL) (positive control) or incubated with *E. coli* K88 1305 and the red algal extract (test). The trans-epithelial electrical resistance (TEER) of the cell monolayer was measured. Secondly, an avian hepatocyte cell line

(LMH) was pre-treated with cell-culture medium only (control) or the red algal extract at three different concentrations (tests). After 24 hours, the cell-culture medium was removed, and cells were inoculated with *E. coli* O78K80 (4.13×10^8 CFU/mL). The percentage of adhesion of bacteria to the cells was obtained by dividing the number of bacteria that adhered to the cells by the total number of bacteria brought into contact with the cells (inoculate). The IPEC-1 cell line incubated with the red algal extract (test) showed an improved gut integrity as observed by a higher TEER when compared to the positive control in the first ten hours of infection with *E. coli* K88 1305. Moreover, the red algal extract reduced the percentage of adhesion of *E. coli* O78K80 at the three concentrations tested compared to the negative control, thus highlighting the capacity of this red algal extract to inhibit bacterial adhesion to the epithelial cells. The studies proved the capacity of this red algal extract to support *in vitro* epithelial barrier function by preventing bacteria from adhering and colonizing target tissues. The in-feed combination of this red algal extract with an immunomodulating green algal extract further showed optimized health parameters and performance of birds raised in commercial conditions.

Key Words: immunity; algae; gut barrier function; polysaccharides

Animal Well-Being and Behavior

221 Plant-based 1,25(OH)₂D₃-glycosides with or without natural antioxidants reduce bacterial lameness in broiler.

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Bacterial chondronecrosis with osteomyelitis (BCO) is caused by the translocation of potentially pathogenic bacteria across the respiratory and intestinal tract, which colonize microfractures in the bone, especially in the femur and tibial heads in broilers. This leads to necrosis of the bone and subsequent lameness. Reducing oxidative stress can increase gut integrity, whereas supporting calcium metabolism improves bone structure. Therefore, a herbal source of the metabolically active form of vitamin D (1,25 dihydroxycholecalciferol-glycosides [G-1,25(OH)₂D₃]) and phytogetic antioxidants were tested in a broiler lameness challenge model. A total of 1560 day-old male Cobb500 were divided into 6 experimental groups with 4 replicates for each: T1- the infection source (IS; 2 replicates; wire floor); T2- negative control; T3- 10 µg G-1,25(OH)₂D₃/kg; T4- 1 µg G-1,25(OH)₂D₃/kg; T5- 1 µg G-1,25(OH)₂D₃/kg plus 500 mg/kg of a polyherbal gut health product; T6- 1 µg G-1,25(OH)₂D₃/kg plus 750 mg/kg of a polyherbal antioxidative and anti-inflammatory product; and T7- 1 µg G-1,25(OH)₂D₃/kg plus 500 mg/kg Thyme oil. All animals were fed the experimental diet on top of a standard corn/soy-based basal diet. The nutritional composition of the basal diet was according to breeder standards. Experimental diets (as a 2-phase program) were fed during the whole trial period of 60 days. Birds (apart from IS) were kept on litter, therefore, T3 to T7 were compared to T2. Incidence of lameness was recorded daily. Birds diagnosed as lame were sacrificed and tibial and femoral lesions in both legs were assessed. Results were analyzed using GLM (R_x64 2.2.4). Cumulative lameness in IS and T2 was highest but did not differ between the two groups (P=0.64). This is in contrast to the supplemented diets, which all showed a lower incidence of lameness compared to T2 (p < 0.001). Within the supplemented treatments, cumulative lameness in T3 and T5 was higher than in the other treatments, with T7 showing the lowest values. Birds fed 10 µg G-1,25(OH)₂D₃/kg (T3) had a higher cumulative lameness than those receiving 1 µg G-1,25(OH)₂D₃/kg (T4) (P=0.033). The reduction in cumulative lameness at D60 from T2 to T3 was 83% and from T2 to T7 54%. All supplemented diets reduced the severity of tibial and femoral lesions. This trial showed that the inclusion of a herbal source of G-1,25(OH)₂D₃ with or without the addition of phytogetic antioxidants reduced bacterial lameness through 60 days for broilers. The addition of

antioxidants did not show synergistic effects. Further trials need to be conducted to examine whether the effects of G-1,25(OH)₂D₃ are based on improved bone architecture or the potential effects of G-1,25(OH)₂D₃ on the immune system.

Key Words: oxidative stress; gut barrier; osteomyelitis; *Solanum glaucophyllum*; chondronecrosis

222 Comparative differences of 6-week old broilers and 65-week old broiler breeders during ventilation shut down with heat.

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In 2015, the US Poultry Industry weathered one of the worst highly pathogenic avian influenza (HPAI) disease outbreaks in history resulting in the depopulation of 50.4 million turkeys, laying chickens and other mixed poultry. This HPAI outbreak illuminated the need for timely depopulation methods during an epidemic. Studies were conducted to determine the effectiveness and physiological response of Ventilation Shut down plus heat (VSD+) on different age broiler chickens. The addition of heat to VSD in laying hens drastically decreased the time to death and lowered stress markers in broilers. Using that data, the American Veterinary Medical Association included VSD+, in the depopulation decision tree as a method dependent upon resources and timeliness in 2019. Given animals' response to stress changes with age and environmental conditions, data from the VSD+ studies with the addition of heat, physiology parameters of 6-week old broilers versus 69-week old broiler breeder hens were examined. The simulation chambers allowed for 3.4 ft³ that represents the space volume/hen under industry depopulation conditions. Brain activity was monitored by affixing electroencephalogram electrodes to non-anesthetized birds and the EEG was used to determine time of death (TOD). VSD+ was repeated four times with each bird type. Cloaca temperatures were taken before and after the procedure. Blood was drawn pre- and post- VSD+ to determine corticosterone (CORT), and blood chemistry parameters. Upon death, brain and liver tissue were collected and preserved in RNALater® to determine heat shock protein 70 mRNA expression. The analysis of the treatment differences was accomplished using one-way ANOVA through JMP Pro 14, alpha was 0.05. Despite a significant differences in body weight between the broiler breeders and broilers (P=0.0086), no differences in CORT levels or HSP70 in the brain were observed. However, broiler breeder liver HSP70 was significantly greater (0.611 pg/ml) than 6 wk broilers (0.492 pg/ml; P=0.05). TOD tended to be

quicker for the broilers at 75 minutes and broiler breeders at 113.5 minutes ($P=0.091$). Broiler breeders have a greater expression of HSP70 in the liver compared to broilers while also having less post-CORT. Bird type/age appears to play a more important role in CORT values and HSP70 relationships and thereby heat tolerance more so than body weight. Further studies are needed to observe this relationship in successive time points to verify points of fluctuations.

Key Words: Corticosterone; Depopulation; Ventilation Shut Down Plus; Acute Heat Stress; Heat Shock Protein 70

223 Serum lactate as a physiological indicator of broiler welfare. Aaron Stephan^{1*}, Curtis Leyk¹, Jack Tieberg¹, ¹*Once Innovations, Chanhassen, Minnesota, United States.*

Outcome-based measures of broiler welfare often require subjective scoring by trained personnel and rely on crude outcomes such as feather quality or scoring wound or gait impairments. Additional methods to quantify broiler welfare are accessible to the research community such as fear or stress-induced behavior tests and blood indicators of stress, such as Heterophil:Lymphocyte (H:L) ratios and corticosterone levels. However, these measures require a significant effort and associated cost and are therefore not feasible to be used as an audit tool in commercial applications. Stress activates the adrenergic pathway, which results in secondary elevations in lactate. Given the documented effects on the long-term metabolism of humans, other mammals, and reptiles resulting in chronic stress hyperlactatemia, we hypothesized that broilers housed in different environments may have measurable differences in blood lactate (lactic acid). To test this hypothesis, we first conducted an experiment where broilers were raised under commercial conditions using two light recipes: a standard white-light recipe and a second light recipe shown to reduce stress biomarkers and fear. H:L ratios were measured as well as five behavioral assays of fear and stress. Serum lactate was quantified via colorimetric microplate assay (Sigma Milipore Lactate Assay Kit II) and subsequently by a portable lactate meter using test strips (Nova Biomedical Lactate Plus). Serum lactate was detectable in a range from 0.3-25 mmol/L. Individual serum samples showed a significant correlation (linear regression analysis, $p<0.001$, R version 4.0.3) between the microplate assay and portable lactate meter values. Broiler chickens grown under the low-stress light recipe resulted in statistically significant reductions (average 8.003 vs 9.505 mmol/L) ($p=0.00044$, Student's t-test, R version 4.0.3) in serum lactate compared to broilers grown under standard light recipe conditions. The Hypothalamic-Pituitary-Adrenal (HPA) axis was induced via repeated injection of dexamethasone, the resulting serum lactate levels were measured and analyzed via two-way ANOVA with Tukey's post-test considering dexamethasone treatment and light treatment as factors (R

version 4.0.3). The interaction between dexamethasone and light treatment was significant ($p<0.001$), suggesting that the HPA stress response may partially counteract stress-induced lactate. The advantages of using serum lactate as an indicator of welfare thus include speed (<1 minute per sample), low cost ($<\$2$ per sample), and a highly quantitative nature. Further exploration of lactate as an indicator of broiler welfare is warranted.

Key Words: broiler chickens; fear; stress; lighting; outcome-based welfare

224 Rearing experience and genetic strain affect fear response in laying hen pullets. Ana Rentsch¹, Alexandra Harlander¹, Lee Niel¹, Janice Siegford², Tina Widowski^{1*}, ¹*University of Guelph, Guelph, Ontario, Canada,* ²*Michigan State University, Michigan, Michigan, United States.*

Fearfulness is detrimental to laying hens in non-cage housing systems and can reduce productivity and increase injury and mortality. We asked if rearing complexity and genetic strain affect fearfulness in pullets. Four flocks of Lohmann Brown-lite (LB) and Lohmann Selected Leghorn-lite (LSL) were raised in either barren cages (C) or one of three commercial rearing aviaries of low (A1), intermediate (A2), or high (A3) complexity during the brooding period (increasing # perches/platforms). At 14 weeks of age, 688 pullets were exposed in pairs to a two-stage fear test. Pairs were placed into a circular novel arena (NA) marked as 12 zones, a novel food dish in its centre (Stage 1; 10 min). Then, a novel object (NO, coloured tube) was slowly lowered from the ceiling into the food dish (Stage 2; 5 min). Individual locomotion (zone changes) and alert behaviour (upright walking and standing, vocalizing, jumping, flying) in the NA were recorded in 60sec intervals. In the NO test, the initial (active avoidance vs stationary) and secondary responses (approach; in 30sec intervals) were recorded per bird. Locomotion which suggests exploration, and alertness in the NA were analysed by fitting linear mixed models. Reaction to and approach of the NO were assessed by calculating odds ratios (OR) and hazard ratios (HR) respectively. NA test: Pullets raised in C explored less ($p<0.001$) and performed more alert behaviour ($p<0.001$) than A reared pullets. LB pullets showed more alert behaviour than LSL ($p<0.001$). NO test, initial response: LSL were 2.7 times more likely to actively avoid the NO than LB ($p<0.001$). A1 and A2 pullets were 6.4 and 3.3 times more likely than C and 3.6 and 1.9 times more likely than A3 pullets to actively avoid the NO ($p<0.001$). Secondary response: A1 pullets were 2.9 times, A2 3.4 times, and A3 4 times more likely to approach the NO than C pullets ($p<0.02$). Aviary pullets showed fewer fear responses than C in both the NA and the NO test by being less alert and showing a higher likelihood to explore the arena and approach the novel stimulus. Contrary to pullets raised in A1 and A2, both C and A3 pullets were stationary in response to the NO. C pullets stayed away, whereas A3 pullets recovered and approached, which suggests an

adaptive behavioural response and less fearfulness. The two strains behaved differently in both test phases. In the NA LB pullets seemed more fearful, shown through more agitation. In the NO test, LB and LSL pullets displayed different initial fear responses with no difference in the secondary response. In conclusion, the complexity of rearing housing reduces fearfulness in laying hen pullets, while the effect of genetic strain seems dependent on the frightening stimulus.

Key Words: Welfare; Laying Hen; Fearfulness; Pullets; Housing

225 The delivery matrix, number of chickens and age influence the behavior of broiler chickens during preference tests. Victoria Philp¹, Geraldine Muñoz¹, Paloma Cordero¹, Sergio Guzmán-Pino^{1*}, ¹*Universidad de Chile, Santiago, Metropolitana, Chile.*

Previous studies have been conducted to determine the gustatory preferences of broiler chickens for some sapid compounds. However, there are no studies that analyze the chicken's preferences for compounds added in-water and in-feed simultaneously, as well as no tests that take into consideration the size or ages of the testing flock. The aim of this trial was to assess the effect of the delivery matrix, number of chickens and age on broilers' behavior when performing choice-tests. A total of 96 one-day old male broiler chickens (Ross 308) were tested in two equal trials with 48 chickens each. Birds were distributed into 32 floor pens with 16 pens containing one chicken per pen, and 16 pens containing two chickens per pen. The experimental period lasted 42 days. From days 7 to 42, pens were

randomly assigned to a 2x2x2x4 factorial design: 2 levels of delivery matrix (water/ground wheat), 2 test subjects (one/two chickens) and 2 ages (7-23 days/26-42 days). In addition, four different sapid compounds were used for preference testing against tap water: monosodium glutamate, L-lysine, sucrose and calcium carbonate. Tests lasted 8 hours and chickens were video recorded for 10 minutes during trials. The time of approach (TA), number of bouts (NB), bout duration (BD) and pecking number (PN) at the drinker/feeder containing the sapid compound was registered. Data was analyzed with ANOVA by using the GLM procedure of SAS, considering the main factors and their interactions in the model. The TA, NB, BD and PN were higher ($p < 0.001$) when were 2 chickens per pen. Also, birds showed more activity for these parameters in the 7-23 days than 26-42 days period ($p < 0.001$). Otherwise, the delivery matrix only influenced the NB ($p < 0.001$) and PN ($p < 0.001$). Nevertheless, on the interaction effect between age x chicken number x matrix, significant differences were observed in 7-23 days x 2 chickens x ground wheat fed showing higher TA, NB, BD and PN ($p < 0.001$) among other types of pens. Compounds used for preference testing did not affect any parameters evaluated ($p > 0.328$). In conclusion, broilers' behavior during preference tests is influenced by age and delivery matrix, as well as by the number of chickens performing the tests. A ground wheat matrix and two chickens per pen seems to better adjust to this type of trials.

Key Words: broiler chicken; behavior; chicken number; delivery matrix; preference test

Physiology and Reproduction

226 Pullet genetic strain and rearing housing influence *in vivo* skeletal strains during physical activity.

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Bones are dynamic structures which sense mechanical strain (deformation) and respond by forming or removing bone to maintain structural integrity. We aimed to determine whether a pullet's genetic strain and history of physical activity, determined by rearing housing complexity, affects the mechanical strain stimulus engendered at the tibiotarsal midshaft. Across three consecutive flocks, a total of 7,128 Lohmann Brown-Lite (B) and LSL-Lite (W) pullets were reared in either conventional rearing cages (CC) or two aviary rearing systems of low (L) or high (H) complexity until 16 woa. We sampled 42 birds ($n=7/\text{strain/housing}$) and surgically instrumented their right tibiotarsi with strain gauges at the anterior, medial, and posterior midshaft surfaces. Strains were recorded during ground (GL), uphill (UL), and downhill (DL) locomotion, perching (P), jumping (J), and aerial transition landing (TL), and measures of principal tensile (e_t) and compressive (e_c) strains, and anterior shear strain (shear) were derived. Data was analyzed by ANOVA and post-hoc Benjamini-Hochberg testing, with significance at $p \leq 0.05$. Genetic strain affected anterior (GL), medial (GL, UL, DL), and posterior (GL, DL) axial strains, e_t (DL, J, TL), e_c (G), and shear (GL, UL, DL, TL). Among CC pullets, W had higher medial axial strains compared to B during J. We also saw higher derived strains in W compared to B within housing groups: e_t (in CC during GL; in H during DL and J), e_c (in L during P), and shear (in L during DL). Rearing housing affected anterior (GL, UL, DL) and posterior (TL) axial strains, e_t (GL and J), and there was an interactive effect of genetic strain and housing on e_c (DL, P) and shear (GL, J). In B birds, H had higher posterior axial strains than L (during P). In W, CC and H had higher e_c than L (during P). Across all activities sampled, axial strains averaged $346\mu\epsilon$, $18\mu\epsilon$, and $481\mu\epsilon$ in B and $74\mu\epsilon$, $-867\mu\epsilon$, and $-1164\mu\epsilon$ in W, at the anterior, medial, and posterior sites respectively. Principal tensile and compressive strains averaged $529\mu\epsilon$ and $-876\mu\epsilon$ for B and $953\mu\epsilon$ and $-883\mu\epsilon$ for W. Anterior shear strains were on average $733\mu\epsilon$ for B and $784\mu\epsilon$ for W. In conclusion, genetic strain and rearing housing affect strain values at the pullet tibiotarsal midshaft. We observed higher strain magnitudes in white-feathered compared to brown-feathered birds. We previously reported that aviary rearing led to improved bone microstructure¹, and therefore expected CC to have higher *in vivo* strains compared to

aviary-reared pullets. This was observed in W but not B. These observed differences between genetic strains likely influences bone mass and fracture susceptibility. 1) Vitienes et al. PSA 2021

Key Words: Genetic strain; Skeletal mechanobiology; Bone adaptation; Mechanical strain; Rearing housing

227 Characterizing the eukaryotic ileal microbiota of laying hens house in conventional and cage-free housing types.

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The egg industry is shifting towards cage-free (CF) housing to appeal to changing consumer preferences. However, the potential impact of this shift on hen health and physiology is not well characterized. Previous results from our group reported differences in the bacterial ileal microbiota across housing type and ileal morphology, but not intestinal permeability. Here, we present the first characterization of the eukaryotic ileal microbiota of layer hens using an 18S rRNA gene amplicon sequencing approach, as well as associations with intestinal health parameters. Ileal digesta was collected from hens at a single commercial site across several rooms. DNA was extracted from the digesta of 32 hens housed in conventional cages (CC), and 48 hens housed in CF using the Qiagen Powerlyzer Powersoil kit. Following amplification of the V9 region of the eukaryotic 18S rRNA gene, DNA was sequenced on the Illumina MiSeq platform. Paired end reads were merged, and quality filtered in Mothur v1.43.0, followed by *de novo* Operational Taxonomic Unit (OTU) clustering at 99% similarity. OTUs were classified with the SILVA SSU v138 reference database, and OTUs classified as vertebrates, plants, and arthropods or with less than 10 reads were removed. Analysis resulted in 3,136,400 high quality reads across 1,370 OTUs. Pearson correlations were calculated between OTUs and intestinal parameters, and between eukaryotic and prokaryotic microbiota. The eukaryotic ileal microbiota of layer hens was predominantly composed of two genera of yeast, *Kazachstania* (77.1%) and *Saccharomyces* (9.7%), as well as the parasitic taxa *Eimeria* (7.6%). No single OTUs were differentially abundant after correcting for false discovery ($P > 0.05$; $q > 0.1$), but differences were detected at the whole community level by PERMANOVA over Bray-Curtis distances ($P < 0.001$). *Eimeria* was positively correlated with *Clostridia* abundance in the CF group and negatively correlated with *Saccharomyces* and *Lactobacillus* in the CC group ($r^2 \geq |0.35|$). Two *Kazachstania* OTUs and one *Saccharomycetaceae* OTU were positively correlated with intestinal permeability in the CF group ($r^2 \geq 0.35$). Surprisingly, 15 OTUs classified as *Eimeria* were negatively correlated with intestinal permeability ($r^2 \leq -0.35$). While these results yielded many expected relationships, novel and unexpected associations

were identified suggesting that disease challenge studies provide limited understanding of the complexities associated with host-microbe and microbe-microbe interactions.

Key Words: conventional cage; cage free; microbiome; laying hens; housing type

228 Transcriptomic responses of vagina and utero-vaginal junction of the turkey reproductive tract after artificial insemination. Sunantha Kosonsiriluk¹, Kent Reed¹, Sally Noll¹, Ben Wileman², Kahina Boukherroub^{1*}, ¹*University of Minnesota, Saint Paul, Minnesota, United States*, ²*Select Genetics, Willmar, Minnesota, United States*.

The turkey industry relies on artificial insemination (AI) to produce fertile eggs. Sperm enter through the vagina, then move to the utero-vaginal junction (UVJ), where they reside in sperm storage tubules (SSTs), before traveling to the infundibulum where fertilization occurs. The proximal segment of the oviduct plays a key role in sperm selection, transport, and storage affecting long term fertility of turkey breeder hens. The aim of the study is to characterize transcriptomic responses of the vagina and UVJ of virgin and inseminated turkeys. We hypothesized that AI differentially alters the transcriptome of the vagina and the UVJ to support their respective functions of sperm selection and storage. Vagina and UVJ tissues were collected from 30-week-old hens, 48h after their first AI, and age-matched virgin controls (n=4 per group). Transcriptomes of the vagina in relation to the UVJ were examined in virgin and AI groups using RNA sequencing. Total RNA was extracted using Trizol reagent, libraries were constructed using the TruSeq Stranded mRNA kit, and sequenced on Illumina

NovaSeq 6000. Differential expression analysis was performed through pairwise comparisons between vagina and UVJ of virgin and AI birds. A total of 498 differentially expressed genes (DEGs) were identified in the virgin group while 899 DEGs were identified in the AI group (q -value < 0.05 and log2 fold change > 2). Enriched biological processes between the two tissues in both groups were determined with ShinyGO v0.75. Gene Ontology enrichment analysis of commonly expressed genes between vagina and UVJ in both groups involve basic cellular, metabolic, and biosynthesis processes. The carbonic anhydrase (CA activity) pathway was uniquely identified in the UVJ, and expression of a number of genes (*CA2*, *CA3B*, *CA4*, *CA7*, and *CA8*) increased after AI. Carbonic anhydrase is present in cells of avian SSTs and plays a role in acid-base homeostasis which affects sperm storage and motility. By contrast, *a response to stimulus* and *neuropeptide signaling* pathways were enriched in the vagina of AI birds. Vasoactive intestinal peptide (VIP) expression was detected after AI and its expression was greater in the vagina compared to the UVJ. VIP may play a role in the local control of smooth muscle motility at the entry of the reproductive tract. The enriched pathways of the vagina suggest mechanisms of sperm selection and transport while UVJ expression favors sperm storage and motility. Further studies are underway to investigate how these pathways change in relation to the age of the hen and fertility. Understanding the process of sperm entry, transport, and storage will advance the knowledge towards increasing fertility in turkey breeder hens.

Key Words: turkeys; transcriptome; artificial insemination; uterovaginal junction; vagina

Metabolism and Nutrition, Enzymes II

229 Effects of limestone solubility, dietary calcium content and dose level of a novel consensus bacterial 6-phytase variant on growth performance, mineral digestibility and utilization in broilers. Wenting Li^{1*}, Yueming Dersjant-Li¹, Cees Kwakernaak², Vanessa Lagos², Leon Marchal¹, Abiodun Bello¹, ¹*Danisco Animal Nutrition & Health, Wilmington, Delaware, United States*, ²*Schothorst Feed Research, Lelystad, Netherlands*.

Effects of limestone solubility, dietary calcium (Ca) and a novel consensus bacterial 6-phytase variant (PhyG) on broiler growth performance, bone ash and Ca and phosphorus (P) digestibility were investigated. A completely randomized block design with a 2x2x2+1 arrangement incorporated 2 limestones (high soluble [HSL], 94%; low soluble [LSL], 58% soluble after 5 min at pH3), 2 Ca levels: Medium and High (starter [St]: 0.80, 0.95%; grower [Gr]: 0.70, 0.85% and finisher [Fn]: 0.60, 0.75%), and 2 PhyG levels (500 and 1,500 FTU/kg). Basal diets, based on corn, wheat, SBM rapeseed meal and sunflower meal, contained digestible P (0.22, 0.19, 0.15% for St, Gr and Fn, respectively) that were lower than recommended levels. One Ca and P diet (PC) that met the nutrient recommendation, without phytase, was included with 0.95%, 0.85% and 0.75% Ca and 0.39, 0.36 and 0.32% digestible P for St, Gr, Fn, respectively. Diets were fed to day-old Ross 308 males (pen=6, 30 birds/pen, St: 0 to 10 d, Gr: 11 to 20 d, Fn: 21 to 35 d). Ileal digesta was collected at d 20 to determine apparent ileal digestibility (dig) of Ca and P. Tibias were collected on d 10, 20 and 35 for ash determination. Data were analyzed by one-way ANOVA to compare the responses vs. PC and by factorial analysis with PC excluded. Overall performance (BWG, FI, FCR, bone ash) was not different between PC and 1500 FTU/kg diets. FCR was not different among all diets at 35 d (ave.=1.42). The P dig was 22 or 45% higher with 500 or 1000 FTU/kg phytase inclusion, respectively vs. PC ($P<0.05$). Regarding the diet factors, there were interactions between Ca and phytase ($P<0.05$) and solubility and phytase ($P<0.05$) on final BWG. Lower BWG was seen with High Ca or HSL, whereas no difference was observed at 1500 FTU/kg. Tibia ash was not affected by solubility of Ca at d 35. Phytase at 1500 FTU/kg increased tibia ash ($P<0.05$) vs. 500 FTU/kg at all ages. Limestone solubility *per se* did not affect P dig among phytase diets, rather, it was reduced by High Ca (66.0%) vs. Medium Ca level (70.5%, $P<0.05$). On the contrary, solubility rather than Ca level impacted Ca dig, where higher Ca dig was seen with HSL (59.9%) vs. LSL (55.2%, $P<0.05$). Phytase at 1500 FTU/kg increased both Ca and P dig irrespective of limestone or Ca level ($P<0.05$), which was 7 (59.5 vs. 55.7%) or 19% (74.1 vs. 62.4%) higher compared to 500 FTU/kg for Ca and P dig, respectively. In conclusion, the inclusion of high dietary Ca levels and the use of highly soluble limestone appear to be detrimental to bird performance, however, inclusion of high

levels of phytase prevented the negative effects of level and solubility of Ca, and maintained growth performance.

Key Words: digestibility; performance; phytase; ash; calcium and solubility

230 A new phytase efficient over a wide range of dietary phytate-phosphorus in 0-to-21-day broilers. Maamer Jali^{1*}, Michael Kidd², Pierre Cozannet¹, Baris Yavuz¹, Marcio Ceccantini¹, Aurélie Preynat¹, Estelle Devillard¹, ¹*Adisseo France S.A.S., Center of Expertise in Research and Nutrition, Malicorne, France*, ²*University of Arkansas, Fayetteville, United States*.

An experiment was carried out to investigate the effects of varying levels of phytate-phosphorus (PP) and a novel thermostable bacterial 6-phytase on growth performance and bone mineralization of broilers fed corn-soybean meal-based diets from 0 to 21 days of age. A total of 2,400 one-day-old male Ross 308 chicks were allocated into 12 treatments in a randomized complete block design with a 3 x 4 factorial arrangement of increasing levels of dietary PP (0.25%, 0.34% or 0.43%) and doses of phytase (0, 500, 1,000 or 2,000 FTU/kg diet). All treatments had 8 replicate pens with 25 birds per pen. Body weight (BW), body weight gain (BW gain), feed intake (FI), and feed conversion ratio (FCR) were measured on day 21. In addition, tibia samples were collected on day 22 for tibia ash content determination. Increasing dietary PP levels linearly ($P<0.0001$) decreased final BW, BW gain, FI, and tibia ash content and increased FCR. Regardless of dietary PP level, increasing phytase dose from 0 to 2,000 FTU/kg linearly ($P<0.0001$) increased the final BW (1,196 g vs 1,124 g), BW gain (1,151 g vs 1,079 g) and FI (1,345 g vs 1,296 g). Phytase dose quadratically ($P<0.0001$) decreased the FCR from 1.202 to 1.169. There was also a quadratic ($P<0.001$) increasing response with increasing phytase dose on tibia ash content. In conclusion, increasing dietary levels of PP impaired performance and bone mineralization while phytase supplementation counteracted these negative responses of broilers. The absence of interaction between phytase and PP, suggests an efficient activity of the enzyme relative to the substrate, from 500 FTU/kg diet. However, higher doses may speed up substrate degradation and benefit animal performance.

Key Words: Broilers; Phytase; Growth performance; Bone mineralization; Phytate

231 A novel consensus bacterial 6-phytase variant maintained performance and bone quality in broilers fed diets with different phytate P levels without added inorganic P compared to a commercial control diet. Glenmer Tactacan^{1*}, Abiodun Bello¹, Jeff Firman², Amir Ghane³, Yueming Dersjant-Li¹, Leon Marchal¹, ¹*Danisco Animal Nutrition & Health, IFF, Leiden, Netherlands*,

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This study evaluated the effects of a novel consensus bacterial 6-phytase variant (PhyG) in inorganic P free (IPF) corn based mixed diets with two levels of phytate P (PP) as compared to a commercially representative diet containing phytase and xylanase. A total of 900 Ross 308 male day-old chicks were randomly assigned to 3 dietary treatments: 1) corn/soybean meal/wheat (10%)/full-fat soybean-based basal diet containing inorganic P and 0.28% PP (positive control, PC), 2) PC reformulated as IPF diet with 0.28% PP (IPF-LP) and 3) PC reformulated as IPF diet with 0.30% PP (IPF-HP). The analyzed PP levels (average over all phases) were 0.30, 0.30 and 0.33% in PC, IPF-LP and IPF-HP, respectively. The broilers were fed pelleted diets using a 4-phase feeding program: starter (0-10 d), grower (10-21 d), finisher 1 (21-35 d), and finisher 2 (35-42 d). The PC contained PhyG at 1,000 FTU/kg (all phases), while IPF-LP and IPF-HP contained PhyG at 3,000 FTU/kg in starter, 2,000 FTU/kg in grower, and 1,000 FTU/kg in finishers phases. All diets contained xylanase (750 U/kg). PC was applied with full matrix for the combined enzyme (-0.20 to -0.21% points Ca, -0.16 to -0.17% points digestible P, -0.04% points Na, -0.02 to -0.05% points digestible AA, -70 kcal/kg ME) vs breeder's recommendations. A randomized complete block design consisting of 12 replicate pens per treatment with 25 birds per pen was used. The data were subjected to one-way ANOVA with differences between means determined using Tukey's HSD by Fit Model platform of JMP 16. Overall, compared to PC, PhyG supplemented IPF diets at both PP levels produced similar growth performance in all phases except for the difference on average daily feed intake (ADFI) during 1-21 d (IPF-HP diet increased ADFI by 5.9% vs PC; $P<0.05$). The final body weight (BW) at d 42 for IPF-LP (3,012 g) or IPF-HP (2,992 g) was not significantly different ($P=0.218$) to that of PC (2,891 g). Similarly, the 1-42 d mortality corrected FCR for IPF-LP (1.506) or IPF-HP (1.520) was not significantly different ($P=0.215$) to that of PC (1.554). Tibia bone ash at d 21 and 42 was similar between birds fed PC (40.8% and 32.6%) and the IPF-LP (40.9% and 32.1%) and IPF-HP (40.0% and 31.6%) diets. There was no difference on performance and tibia bone ash between the IPF-LP and IPF-HP birds except during the 11-21 and 1-21 d periods where ADFI was increased ($P<0.05$) by 6.9% and 5.9%, respectively. Overall, the results indicated that the supplementation of a novel consensus bacterial 6-phytase variant to IPF diets with analyzed phytate P level at 0.30 or 0.33% maintained growth performance and bone quality compared to a commercially representative diet containing phytase and xylanase.

Key Words: broilers; bone; growth performance; phytase; inorganic phosphorus-free

232 A second independent trial showing a novel consensus bacterial 6-phytase variant maintained performance and tibia ash in broilers fed corn-SBM diet without added inorganic P and deficient in nutrients and energy. Leon Marchal^{1,2*}, ¹Abiodun Bello², Jeffre Firman³, Yueming Dersjant-Li², ¹Wageningen University & Research, Wageningen, Netherlands, ²Danisco Animal Nutrition - IFF, Wilmington, United States, ³Missouri Contract Poultry Research, Boonville, United States.

A recent study demonstrated a novel consensus bacterial 6-phytase variant (PhyG) has the potential to replace inorganic P in all feeding phases in broilers fed a corn and soybean meal (SBM)-based vegetable diet (with corn DDGs at 1.5 to 2.2%) when compared to a non-phytase supplemented positive control. In this study, a commercially representative positive control (PC) diet supplemented with 750 FTU/kg of PhyG and 750 U/kg of xylanase (with consideration of digestible AA, ME, Ca, P and Na contribution of these enzymes) was formulated with 22.6, 21.0, 19.6, 19.9% CP; 1.88, 1.08, 0.96, 0.91% digestible Lys, 2900, 3000, 3050, 3100 kcal/kg ME; 0.72, 0.68, 0.61, 0.51% Ca and 0.26, 0.22, 0.18, 0.16% digestible P, respectively in 4 feeding phases (0-10, 10-21, 21-35, 35-42 days) and with 0.15% Na in all phases. An inorganic P free (IPF) diet was formulated with the same nutritional compositions as PC but without usage of any inorganic P or bone meal source and was supplemented with PhyG at 3000, 2000 and 1000 FTU/kg in the starter, grower and finisher 1&2 phase respectively and 750 U/kg of xylanase in all phases. Diets were based on corn, SBM and oil, 1-1.6% DDGS. The IPF diet contained also 0.5% of oat hulls. The average analyzed phytate P contents were 0.29 and 0.31% in PC and IPF diets respectively. Diets were pelleted and fed *ad lib* to 12 replicate pens (Ross 308 male broilers; 25 birds/pen). On day 21 and 42, tibia ash was determined (pooled 4 birds per pen). Data were analyzed by one-way ANOVA using JMP 16. Treatment means were separated by Tukey test. The PC and IPF treatments were not statistically different with respect to body weight, feed intake and mortality corrected FCR in each feeding phase and cumulative phases of 1-21, 1-35 and 1-42 days. Tibia ash were similar for both treatments for day 21 (41.6 and 40.8%) and day 42 (32.21 and 32.89%) indicating that bone mineralization was fully maintained in the IPF diet with the phase dosing of the phytase. As the 2nd independent trial, these findings demonstrate the repeatability of this new phytase to potentially replace inorganic P in all feeding phases in broilers fed a corn and SBM-based vegetable diet. This study established that compared to a commercially representative PC with supplementation of commercial level phytase and xylanase and respective matrix application, the novel phytase maintained performance and tibia ash in corn-SBM diets without added inorganic phosphorus. Though it is appreciated that complete removal of Inorganic P in corn-SBM diets is not commercial practise, understanding the limits of the application helps

broaden the opportunities in a market where feed costs are rising and pressure on sustainability increases.

Key Words: broilers; phytase; sustainability; inorganic phosphorus free; corn-SBM diet

233 Supplementation of a novel consensus bacterial 6-phytase variant in inorganic phosphorus-free diets reduced in energy and nutrients maintained egg productivity and egg quality from 23 to 72 weeks of age.

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The efficacy of a novel consensus bacterial 6-phytase variant (**PhyG**) to improve or maintain egg productivity, eggshell quality, and body weight of laying hens fed inorganic phosphorus (**IP**)-free diets reduced in energy and nutrients from 23 to 72 weeks of age (**woa**) were evaluated. A total of 448 Hy-Line W80 (4 hens/cage × 28 cage replicates per treatment) were randomly assigned to four experimental diets. The diets included 1) an energy and nutrient adequate positive control diet (**PC**), 2) an IP-free negative control diet reduced in ME by 75 kcal/kg, Ca by 0.20%, Na by 0.02%, and dig AA by up to 0.04%-unit vs PC (**NC** contained 0.12% of available P). Other diets were the NC diet supplemented with PhyG at 3) 600 FTU/kg (**NC+600G**) or 4) 900 FTU/kg (**NC+900G**). The diets were formulated to contain corn-SBM with wheat bran and rice bran and were provided to the hens *ad-lib* in the daily 16 hours of light. JMP 15.1 was used for the data analysis and treatment means were separated by Tukey HSD ($P \leq 0.05$ as significant). The overall 23 to 72 woa marketable egg production was decreased ($P < 0.05$) by the NC (93.4%) vs PC (94.5%) however completely alleviated by both NC+600G (95.4%) and NC+900G (96.3%), while the lay rate was improved by NC+900G vs. PC. Overall egg mass and FCR (measured on four weeks basis) were similar between the PC (60.6 g and 1.79) and the NC (60.0 g and 1.80), respectively, however egg mass was improved ($P < 0.05$) by NC+600G (60.9 g) vs. NC and NC+900G (61.8 g) vs. both control diets, while FCR was similar in NC+600G (1.81) and NC+900G (1.79) vs. both control diets. Overall eggshell breaking strength (48 eggs / diet repeatedly measured on four weeks basis) was decreased ($P < 0.05$) in NC (4.53 kgF) vs. PC (4.78 kgF) and was restored by both NC+600G (4.78 kgF) and NC+900G (4.78 kgF). While starting (23 woa) BW was similar ($P > 0.05$) between all treatments, the final (72 woa) BW was decreased ($P < 0.05$) by the NC (1,640 g) vs PC (1,700 g), however fully alleviated by NC+600G (1,701 g) and NC+900G (1,695 g). Overall, 23 – 72 woa feed cost / dozen eggs produced was reduced by NC+600G (0.520 USD) or

NC+900G (0.517 USD) vs. PC (0.545 USD). These findings demonstrated that feeding the IP-free and nutrient and energy-reduced diet to hens decreased productivity, and that application of the 600 or 900 FTU/kg PhyG in the NC diet cost-effectively improved or maintained egg productivity, eggshell quality, and body weight of laying hens. In conclusion, PhyG at 900 FTU/kg demonstrated the most cost-effective replacement of IP in laying hen diet also reduced in Ca, Na, dig AA and energy, with fully compensated egg production and quality through 23 – 72 woa laying cycle.

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

234 Effects of increasing dose levels of a novel consensus bacterial 6-phytase variant on apparent total tract digestibility of calcium and apparent ileal digestibility of phosphorus and sodium in broilers: meta-analyses.

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Using a meta-analysis approach, the effects of increasing dose of a novel consensus bacterial 6-phytase variant (**PhyG**) were evaluated on apparent total tract digestibility (**ATTD**) of Ca and apparent ileal digestibility (**AID**) of P and Na in broiler. In total, 378 datapoints from 12 datasets (samples collected during d 18-21 for ATTD Ca and at d 21 for AID P) were used in the meta-analysis of ATTD Ca and AID P, and 444 datapoints from 14 datasets (samples collected at day 10, 21 and 35 respectively) were used for AID Na. The overall datasets used in the study covered different diet forms (mash or pellet); diet types (simple diets to complex diets based on corn or wheat-soy bean meal and without or with secondary ingredients such as rapeseed meal, sunflower meal, broken rice, rice bran, DDGs and wheat bran), and broiler-graded limestone sources to generate globally representative variations in dietary phytate P (**PP**) levels (in the range of 0.20 to 0.39%) and limestone solubility (in range of 42 to 97% at 5 min). With few exceptions, most of the diets were formulated without inorganic P source or M&BM, and the ranges of analyzed Ca, total P, and Na, were 0.5 to 0.85%, 0.37 to 0.61%, and 0.11 to 0.12% respectively. The doses of PhyG tested across all studies were 0 and 4000 FTU/kg and the range of analyzed phytase activities were 192 - 4,860 FTU/kg (above the respective 0 FTU/kg phytase-supplemented diets (**NC**)). The increasing PhyG dose effects were analyzed across respective datasets for each of ATTD Ca and AID P and Na responses using the non-linear curve fitting function of JMP 16.1. Increasing PhyG dose exponentially ($y = \text{Asymptote} + \text{Scale} * \text{EXP}(\text{Growth Rate} * \text{analyzed phytase dose})$) increased ($P < 0.001$) ATTD Ca, AID P and Na. The curve fitting estimates were 39.8, 46.9, 51.0, 54.9, 56.6, 56.8 and 56.9% for ATTD Ca; 43.6, 53.9, 60.3, 66.8, 70.3, 70.8 and 70.9% for AID P; and -140.7, -110.0, -95.1, -84.2, -81.1, -

80.9 to -80.9% for AID Na, at phytase dose levels of 0, 250, 500, 1,000, 2,000, 3,000 and 4,000 FTU/kg, respectively. Based on the studies designed specifically with different PP levels, digestible P (AID co-efficient multiply by analyzed dietary P) release by phytase above NC increased with increasing PP level. Based on the studies specifically designed with different limestone, the high soluble limestone reduced AID P in the NC and at low phytase dose vs. the low soluble limestone. Overall, increasing PhyG dose exponentially increased digestibility of Ca, P, and Na and dietary PP level and solubility of limestone used are major factors to consider in determining phytase dose usage and P, Ca and Na matrices application in broiler.

Key Words: broiler; phosphorus; phytate; limestone solubility; novel consensus bacterial 6-phytase variant

235 Effect of a novel hybrid bacteria 6-phytase at different levels on the performance and bone parameters of broilers fed a corn soybean meal-based diet from 1 to 42 days of age. Nicolas Martinez^{1*}, Adebayo Sokale¹, Peter Ader³, Dan Moore², ¹BASF, Florham Park, New Jersey, United States, ²Colorado Quality Research, Willmington, Colorado, United States, ³BASF, Lampertheim, Germany.

This study evaluated the efficacy of a novel hybrid bacteria 6-phytase (Natuphos[®] E; NPE) on growth performance parameters and tibia bone ash in broiler feed when supplemented at 500, 1000 and 1,500 FTU/kg. A total of 840 male broilers (21 birds × 8 pens/treatment) were randomly allocated to each of five dietary treatments in a randomized complete block design. The five diets tested in this study were 1) a nutrient adequate diet (18 - 22% CP, 3040 - 3166 Kcal/kg ME, 0.88 - 0.72% Ca, and 0.44 - 0.36% available P; PC); 2) a diet reduced by 0.15 % in available P and 0.16 % in Ca, only (NC); 3) NC with 500 FTU/kg NPE; 4) NC with 1000 FTU/kg NPE; 5) NC with 1500 FTU/kg NPE. A corn soybean meal-based, all-vegetable diet with 5% DDGs was used in the study. All diets were pelleted and were fed ad libitum over 3 phases: starter (d1 - 14), grower (d14 - 28), and finisher (d28 - 42). Feed intake and body weight were measured at d1, 14, 28, 35, and 42. Body weight gain (BWG), daily feed intake (ADFI), and feed conversion ratio adjusted for mortality (FCR) were calculated accordingly. The left tibia was collected from 16 birds per treatment at d21 for bone ash analysis. All data were analyzed by One-way ANOVA according to Randomized Complete Block Design. Treatment means were further separated using Tukey's HSD test. At 1 - 14, 1 - 28, and 1 - 42 days, BWG and FCR were significantly lower in the NC in comparison to the PC and all NPE supplemented levels ($P < 0.05$). No significant difference in BWG and FCR was observed between PC and 500 FTU/kg NPE at 1 - 14 and 1 - 28 days ($P > 0.05$). Furthermore, no significant difference in BWG and FCR was observed between 500, 1000 and 1500 FTU/kg NPE and the PC ($P > 0.05$). Cumulatively, at d1 - 42, BWG and FCR were

significantly higher in 1500 FTU/kg NPE in comparison to NC ($P < 0.05$) and numerically higher than the PC. Tibia ash was significantly lower in the NC in comparison to the PC at d21 ($P < 0.05$). However, there was no difference in tibia ash response between PC and all levels of NPE supplementation. In conclusion, this study showed that replacement of 7.2 kg/t monocalcium phosphate (calculated to be equivalent to 0.15% available P) with 500 FTU NPE/kg resulted in growth performance equivalent to PC.

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

236 Evaluation of a new technique to identify formaldehyde in broiler diets and this relationship with the phytase and xylanase recovery. Alexandre de Brito^{1*}, Vanessa Aparecida Caldato dos Santos², Juliana Pulita³, Filippa Scortegagna³, Alexandra Garcia¹, Vinícius Assuena⁴, Gustavo Fonseca⁴, Lisiane Calza⁴, ¹AB Vista Feed Ingredients, Goiania, Goias, Brazil, ²GT Foods, Maringá, Brazil, ³Mercolab, Chapeco, Brazil, ⁴Auster Nutri, Hortolandia, Brazil.

Phytase (PHY), Xylanase (XYL) and formaldehyde-based additives (FBA) are routinely used in broiler nutrition. FBA are used to decrease the incidence of Salmonella in feeds. Though enzymes activity recovered in the feed is the most common way to measure dietary enzyme inclusion, FBA reduce the analyzed enzymes activity in the feed (Santos *et al.*, 2013). The objective of this trial was to evaluate the levels of FBA in diet, in addition to correlating these values obtained by commercial kits, and this effects on enzyme recovery. For this, diets containing 5,000 FTU/kg of PHY (Quantum Blue, AB Vista, Marlborough, UK) and 28,000 BXU/kg of XYL (Econase XT, AB Vista, Marlborough, UK) were produced and sent to the Mercolab Laboratory in Chapecó, Brazil. The diet was divided into 40 samples of Mash diets, subdivided into 10 treatments of 4 samples/treat, as follows: Treat 1 (samples without any processing), Treats 2, 4, 6, 7, 9 (added 1.25 kg/ton of FBA with 30% formaldehyde concentration), Treats 3, 5, 8, 10 (added 2.50 kg/ton of FBA with 30% formaldehyde concentration). The samples were also submitted to a volatilization process by 24 hours in an oven at 35C (Treat 6), or submitted to volatilization at room temperature in 0 (Treat 2 and 3), 24 (Treat 4 and 5), 48 (Treat 7 and 8) or 72 (Treat 9 and 10) hours before the Elisa analysis. All samples, before analysis by Elisa, were evaluated by reflectometric test with strips and reagent by Reflectoquant[®]/Merk. The recovery means were compared by orthogonal contrasts, taking the Treat 1 as the basis for the contrasts. Analyzing the data obtained by kit Reflectoquant[®]/Merk and the volumes of FBA in the diet, resulted in a Pearson Correlation value of R^2 0.691, where it was possible to generate the following equation: FBA = (Reflectoquant[®]/Merk result in mg/L - 11,845)/432,63. The average of the Elisa results were: PHY (Treat 1 - 5303^a, Treat 2 - 3182^b, Treat 3 - 1924^b, Treat 4 - 2747^b, Treat 5 -

2327^b, Treat 6 – 3985^a, Treat 7 – 3292^a, Treat 8 – 1160^b, Treat 9 – 3159^{ab}, Treat 10 – 1496^b FTU/kg); XYL (Treat 1 – 27138^a, Treat 2 – 18438^b, Treat 3 – 15075^b, Treat 4 – 18215^b, Treat 5 – 17013^b, Treat 6 – 19938^a, Treat 7 – 18525^b, Treat 8 – 11238^b, Treat 9 – 23312^a, Treat 10 – 12825^b BXU/kg), where different letters are ($P < 0.05$). The use of FBA brings great pressure to the enzyme recovery process by the Elisa, although it does not affect the animal performance, according to previous work reported by (Santos *et al.*, 2013). One way to mitigate these false negatives evaluations by Elisa, would be to leave the sample for 24 hours in an oven at 35C or even to volatilize formaldehyde for 72 hours at room temperature.

Key Words: Enzyme; Formaldehyde; Elisa; Mitigate; Recovery

237 A low protein – low energy diet fortified with higher doses of a consensus bacterial 6-phytase, and a carbohydrase-protease enzyme complex sustains broiler performance. Amir Ghane^{1* 2}, ¹Danisco Animal Health and Nutrition, Singapore, Singapore, ²University of Guelph, Guelph, Canada.

Exogenous enzymes like phytase as well as xylanase, amylase and proteases, either independently or in combination (XAP), when included in diets at levels greater than the standards followed by the industry are likely to elicit greater responses in terms of their nutrient sparing effects. The present study aimed towards assessing the effects of phytase and XAP at higher than the commercially practised levels of inclusion either alone or in combination on performance and key metabolic indices in broiler chickens fed diets which were reformulated as per the combined matrix of the phytase and the XAP. In a 42-d feeding trial 1100 male broiler chickens (Cobb 500) were fed with diets deficient in apparent metabolizable energy (AME) by 120 kcal/kg and crude protein (CP) by 3%. These diets were supplemented with a *consensus bacterial* 6-phytase (1000 and 2000 FTU/kg) and an XAP (100 and 200 mg/kg) either independently or together. Body weight, feed intake, feed conversion ratio and liveability, digesta viscosity and serum biomarkers (glucose, uric acid and peptide YY) were determined and compared against a nutrient-adequate positive control (PC) and the negative controls (NC1 with nutrient downspec in according to the full matrix of the phytase and the XAP and NC2 with nutrient downspec according to the full matrix of the XAP alone). The data were analysed for independent dose effects of the phytase and the XAP against PC and NC-1 and NC-2. A 3 x 3 factorial design phytase (0, 1000 and 2000 FTU/kg diet) and XAP (0, 100 and 200 mg/kg diet) were used as the grouping factors and data analysed by general linear model of SPSS (version 26.1) to get the main effects and their interactions. Both the phytase ($P = 0.026$) and the XAP ($P = 0.051$) independently improved body weight at harvest linearly with dose. Increasing the dose of XAP improved feed conversion ($P = 0.004$). Combining 1000

FTU/kg phytase with 200 mg/kg XAP yielded body weight and feed conversion close to the PC (significant improvement over the NC groups at $P < 0.05$) and decreased digesta viscosity ($P < 0.05$), uric acid ($P < 0.05$) and peptide YY ($P < 0.05$) in serum. Inclusion of phytase (2000 FTU/kg) and XAP (200 mg/kg) at doses higher than the industry defined standards appeared to sustain performance of broiler chickens with a low CP- low AME diet which should facilitate greater economic return.

Key Words: Phytase; Protease; Amylase; Xylanase

238 Validation of target dosing matrix of a novel hybrid bacteria 6-phytase on the broiler performance and bone parameters from 1 to 42 days of age. Everton Krabbe^{2*}, Caroline Bavaresco³, Nicolas Martinez¹, Adebayo Sokale¹, Peter Ader⁴, ¹BASF, Florham Park, New Jersey, United States, ²Embrapa Swine and Poultry, Brazil, Brasilia, Brazil, ³Federal University of Pelotas, Pelotas, Brazil, ⁴BASF, Lampertheim, Germany.

The validation of an extended matrix of a novel hybrid bacteria 6-phytase (Natuphos[®] E; NPE) was performed in this trial. Apparent metabolizable energy (AME), Ca and P matrices for starter, grower, finisher phase using 750 FTU/kg (85 kcal; 0.43% CP; 0.20% Ca; 0.18% aP), 1000 (90 kcal; 0.51% CP; 0.21% Ca; 0.19% aP), 1500 (90 kcal; 0.66% CP; 0.23% Ca; 0.21% aP) and 2000 FTU/kg (92 kcal; 0.81% CP; 0.24% Ca; 0.22% aP) were evaluated on broiler growth performance and bone characteristics. A total of 2,250 Cobb male broilers (10 birds x 25 pens/treatment) were randomly allocated to 9 dietary treatments in a randomized complete block design, as follows: T1) a nutrient adequate diet with inorganic phosphate (0.43; 0.38; 0.31% aP), Ca (0.92; 0.82; 0.66%), AME (3.10; 3.20; 3.25 Mcal/kg); T2) a negative control (NC) applying 750 FTU/kg matrix reduction (NC1); T3) NC1+ Phytase 750 FTU/kg; T4) NC applying 1000 FTU/kg matrix reduction (NC2); T5) NC2+1000 FTU/kg T6) NC applying 1500 FTU/kg matrix reduction (NC3); T7) NC3+1500 FTU/kg; T8) NC applying 2000 FTU/kg matrix reduction (NC4); and T9) NC4+2000 FTU/kg. All diets were corn-SBM based and fed ad libitum over 3 phases: starter (1-14 days), grower (14-28 days), and finisher (28-42 days). Feed intake and bodyweight were measured at 14, 28, and 42d to calculate body weight gain (BWG), daily feed intake (DFI), and feed conversion ratio (FCR). Data were analyzed by One-way ANOVA according to Randomized Complete Block Design. Treatment means were further compared using Tukey's test. At 14, 28 and 42 days, for each matrix level, BWG was significantly higher in the supplemented group in comparison to their respective NC ($P < 0.001$), with BWG getting back to the level of the overall PC (T1) in all matrix levels. Similarly, FCR at 14 and 42 days were either significantly (T8 and T9) ($P < 0.001$) or numerically (T2 - T7) improved in each matrix level supplementation compared to their respective NC with performance getting back to the level of the overall PC (T1) in all matrix levels.

At 28 days, a decrease in the tibia breaking strength (kgf) and tibia ash (%) was observed for Phytase unsupplemented treatments (NCs) in each adopted nutritional matrix level compared to their respective PC ($P<0.001$). All Phytase supplemented treatments were similar to the overall PC (T1) at all matrix levels. In conclusion, this study validates the matrix values for Natuphos E in broiler chickens at 750, 1000, 1500, and 2000 FTU/kg.

Key Words: chicken; enzyme; nutritional matrix

239 Effect of formaldehyde on phytase recovery, nutrient digestibility and growth performance of broilers fed phytase-containing diets from 1 to 28 days.

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Formaldehyde (FOR) is a potent antimicrobial widely used to reduce bacterial contamination especially *Salmonella* sp. in animal feed. However, FOR can bind amino acid groups resulting in decreased bioavailability and therefore negatively impacting animal performance. This study evaluated the effect of 2 levels of formaldehyde applied to a typical corn-SBM broiler diet containing a novel hybrid bacteria 6-phytase (Natuphos E[®]; NPE) supplemented at 750 FTU/kg on phytase recovery, ileal digestibility (AID), and growth performance. A total of 1,125 one-day-old male Cobb broilers (25 birds \times 15 pens/treatment) were randomly allocated to each of 3 dietary treatments in a randomized complete block design. The 3 diets tested were T1-Control: basal diet (BD) + 750 FTU/kg; T2: BD + 750 FTU/kg + 450 ppm FOR; T3: BD + 750 FTU/kg + 900 ppm FOR. All diets were pelleted and fed ad libitum over 2 phases: starter (1-14 days) and grower (14-28 days). Body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and amino acid digestibility coefficients were analyzed by One-way ANOVA according to Randomized Complete Block Design. Treatment means were further compared using Tukey's test. Phytase recovery was unaffected by the addition of 450 ppm of FOR. However, the addition of 900 ppm of FOR decreased phytase recovery in feed by 16%. At 1-14 days and 1-21 days there were no significant effect of 450 ppm of FOR on BW, BWG and FCR ($P>0.05$). Formaldehyde level at 900 ppm significantly affected BW ($P<0.01$), BWG ($P<0.01$) and FCR ($P<0.001$) at 1-14 days, and BW ($P<0.01$), BWG ($P<0.01$) and FCR ($P<0.01$) at 1-21 days. At 1-28 days, there was no significant effect of 450 ppm of FOR on BW, BWG, and FCR ($P>0.05$), but 900 ppm of FOR significantly increased FCR ($P<0.01$), but not BW and BWG. Furthermore, at 28 days, ileal digestibility of lysine was significantly reduced by the addition of 900 ppm of FOR in comparison to the control group. In conclusion, formaldehyde supplementation at the recommended dose level of 450 ppm did not affect phytase (NPE) recovery,

growth performance and AID of lysine, but dose at 900 ppm affected these variables up to 28 days of age.

Key Words: chicks; enzyme; additives; formaldehyde

240 Microbiota changes due to multicarbohydase and phytase complex and DDGS diets in broiler chickens.

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Distillers dried grains and soluble (DDGS) has a high concentration of non-starch polysaccharides (NSP) especially insoluble ones. NSPs can negatively affect the microbiota. However, NSP can be broken down by exogenous enzymes producing oligosaccharides that are used by the bacteria, acting as prebiotic. Thus, supplementation of enzymes in diets formulated with DDGS may allow the use of this ingredient for broilers, and modulate the intestinal microbiota. A total of 288 day-of-hatch broiler chicks were allocated to six treatments with eight replicates each. The treatments differed by the inclusion of corn-DDGS in the diet (0, 7 and 14%) and by the inclusion (on top) or not of a multicarbohydase (xylanase, arabinofuranosidase and β -glucanase) and phytase complex (MCPC) using a completely randomized 3x2 factorial arrangement (3 DDGS % \times 2 MCPC inclusion). Feed conversion (FC) was calculated at d14 and 28. Samples of ileal and cecal content were collected on d14 and 28 for microbiota analysis. Bacterial DNA was submitted to high-throughput sequencing of the 16S rRNA V3/V4 region. Alpha (α) and beta (β) diversity indices, and microbial composition were measured using QIIME 2. Diversity and composition data were submitted to analysis of variance and compared using Friedman and Kruskal-Wallis test ($P<0.05$). Supplementation of MCPC improved the FC of broilers fed diets with 14% corn-DDGS at d14, and of fed diets with 7% corn-DDGS at d28. α -diversity of ileal and cecal microbiota were affected by corn-DDGS at d14, but not on d28. The inclusion of corn-DDGS reduced the α -diversity of the ileal microbiota, but increased cecal α -diversity at d14. β -diversity of ceca, at d14, and ileal microbiota, at d28, was affected by the treatments (Unweighted UniFrac). At d28, MCPC supplementation reduced the abundance of Proteobacteria in the ceca. An interaction of inclusion of corn-DDGS by MCPC was observed on the microbiota composition. At d14, broilers fed with 7% corn-DDGS+MCPC presented a reduction of Lachnospiraceae family, and an increase of Lactobacillaceae in the ileum. Corn-DDGS inclusion influenced the abundance of Erysipelotrichaceae and Clostridiaceae family in the ceca at d14, and the abundance of Enterococcaceae, Ruminococcaceae and Lachnospiraceae in the ileum at d28. MCPC supplementation reduced Bacillaceae and

Enterobacteriaceae families, and increased Coriobacteriaceae predominance in the ceca at d28. Beyond the effect on nutrient digestibility, MCPC supplementation effect on the performance of broilers fed different levels of

Corn NSP may be related to a better microbiota diversity and composition.

Key Words: gut health; phytase; gut microbiota; carbohydrases; non-starch polysaccharides

Metabolism and Nutrition, General Nutrition

241 Effect of beak trimming at hatch and the inclusion of oat hulls in the diet on egg production, exploratory pecking behavior, particle size preference, and excreta quality of brown-egg laying hens from 19 to 45 weeks of age. J. Ben Mabrouk¹, Gonzalo Mateos^{1*}, A.F. de Juan¹, L. Aguirre¹, L. Cámara¹, ¹*Universidad Politécnica de Madrid, Madrid, Spain.*

The influence of infrared beak treatment (IRBT) at hatch and the inclusion of oat hulls (OH) in the diet on egg production, egg quality, feed preference for coarse particles, exploratory pecking behavior, and excreta quality, was studied in brown egg-laying hens from 19 to 45 wk of age. The control diet was based on corn, wheat, soybean meal, and sunflower meal, and contained 16.7% CP, 0.76% digestible Lys, and 4.5% CF. The diet was presented in mash form and had a GMD \pm GSD of 1,255 \pm 2.15. The experimental diet was obtained by diluting (wt:wt) the control diet with 3% OH. The experimental design was completely randomized with 4 treatments arranged as a 2 \times 2 factorial with IRBT (sham vs. treated) and OH inclusion (0 vs. 3%) as main effects. Each treatment was replicated 18 times and the experimental unit was a cage with an individual hen. Feed intake, egg production, egg weight, and FCR were determined by period (4 wk) and for the entire experiment. Egg quality traits (percentage of broken and shell-less eggs, proportion of albumen, yolk, and shell of the eggs, shell thickness, shell strength, and Haugh units) were measured at the same ages. Particle size preference was determined at 30 wk of age, as the difference in size (GMD \pm GSD) of the original feeds supplied at 08.00 am (lights on) and the refusals remaining in the feeders 4 hours after feed provision. The exploratory pecking behavior was evaluated for 11 consecutive days (42 and 43 wk of age) as the percentage of hens that pecked in less than 5 seconds, a ballpoint pen used as stimulus. Excreta quality was evaluated, based on a visual score of 0 to 3, with a value of 0 representing normally formed excreta and 3 corresponding to watery and very sticky excreta by 3 independent observers blind to treatment. In addition, the DM content of freshly voided excreta dried in an oven at 60 °C for 72 h was determined. The data were analyzed using the MIXED procedure of SAS. No interactions between IRBT and OH inclusion were detected for any of the traits studied and therefore, only main effects are presented. Egg production, egg weight, and egg mass were not affected by treatment. Shell strength was reduced in eggs from birds fed 3% OH (5.49 and 5.08 g/cm²; $P < 0.01$). The preference for coarse particles was greater for the sham than for the treated hens ($P < 0.001$). Beak trimming ($P < 0.001$) and OH feeding ($P < 0.05$) reduced the pecking activity of the hens. Excreta quality score was better and excreta DM content was higher in beak trimmed birds than in sham birds ($P < 0.001$). In summary, IRBT affected particle size preference of the birds but not egg production. Both beak trimming and

OH feeding, reduced the exploratory pecking behavior of the hens.

Key Words: oat hulls; excreta quality; feed particle size preference; infrared beak treatment; layer pecking behavior

242 Nitrogen and phosphorus reduced diets in laying hens – Influence on laying performance, egg quality and nutrient excretion. Carolin Adler^{1*}, Jochen Krieg², ¹*University of Saskatchewan, Saskatoon, Saskatchewan, Canada,* ²*Chamber of Agriculture of North Rhine-Westphalia, Bad Sassendorf, North Rhine-Westphalia, Germany.*

The study aimed to investigate how a reduction of dietary nitrogen (N) and phosphorus (P) affects the laying performance, egg quality, and N and P excretion of Lohman Brown (LB) and Lohman Selected Leghorn (LSL, Lohmann Tierzucht, EW-Group, Visbeck, Germany) layers. A total of 1,008 (50% LSL, 50% LB) 18 wk old laying hens were allocated to 28 enriched cages, resulting in 4 repetitions for the control diet (C) and 5 repetitions for the experiment diets 1 (E1) and 2 (E2), respectively. Each cage comprised 36 animals (900cm² per hen), and the laying period lasted 13 months. Treatments were randomly assigned to the cages. Diets were calculated to contain a decreasing N and P concentration C>E1>E2 (weighted average N and P concentrations (g/kg 88% DM) C: 26.1/4.5; E1: 25.1/4.2 E2: 24.6/3.9). The concentration of Lysine, Methionine + Cysteine, Threonine and Tryptophan were kept at a constant level, and diets were calculated to be isoenergetic. Chemical analyses confirmed the calculated N and P concentrations. Egg production and mortality were documented daily. To evaluate the feed consumption and feed conversion rate, feed back weighs were carried out monthly. Egg weights, shell breaking force, albumen height and Haugh units were measured in the middle and the end (7 and 13 months) of the laying period using 32 (C) to 40 (E1 and E2) eggs per treatment. Data were analyzed using ANOVA (PROC MIXED, SAS 9.4). Diet, breed and interactions were considered as fixed effects, leading to the model: $y_{ij} = \mu + \text{diet}_i + \text{breed}_j + (\text{diet} \times \text{breed})_{ij} + e_{ij}$. P-values and least square means are given in brackets. Performance traits of animals receiving E1 did not differ from C and E2. Layers fed with E2 showed a lower average egg production ($P=0.029$; E2: 90.2 vs C: 92.2%) and lighter egg weights ($P=0.038$; E2: 61.7 vs C: 62.4 g) compared to C. No differences ($P>0.05$) were observed for feed consumption, feed conversion ratio, and mortality between all diets. Breed had no effect ($P>0.05$) on laying performance. Eggshells for LSL broke with less effort ($P<0.001$; LSL: 40.4 vs LB; 46.8 N) compared to LB. Results for albumen height ($P<0.001$; LSL: 9.0 vs LB: 8.3mm) and Haugh units ($P<0.001$; LSL: 94.0 vs LB: 90.3 Haugh units) were higher for LSL compared to LB. Dietary treatments had no effect ($P>0.05$) on egg quality parameters. Compared to C, feeding E1 and E2 reduced both breeds' average N and P excretion by 1%

and 6% and 9% and 15%, respectively. Summarized, E1 can be fed to reduce N and P excretion in LB and LSL layers without negatively affecting laying performance. Further research should be conducted on the effect of N and P reduced feeding strategies on health- and behaviour-based welfare indicators.

Key Words: Feeding strategy; Resource conservation; Environmental protection; Crop-livestock integration; Sustainable agriculture

243 Effects of almond hulls on growth performance, carcass yield, and breast fillet meat quality in broilers raised in floor pens. Jinquan Wang^{1*}, Amit Singh¹, Janghan Choi¹, Brian Bowker², Hong Zhuang², Woo Kim¹, ¹*University of Georgia, Athens, Georgia, United States*, ²*USDA-ARS, Athens, Georgia, United States*.

The objective of this study was to evaluate the effect of graded almond hulls inclusion on growth performance, carcass yield, and breast fillet meat quality of broilers. A total of 640 one-day-old Cobb500 male chicks were randomly placed to four experimental treatments with eight replicates of 20 birds each. Four treatments consisted of a corn-soybean meal control diet and three almond hull treatments containing 2.5, 5, and 7.5% of almond hulls with 97.5, 95, and 92.5 of control diet, respectively. At d14, 28, and 42, feed intake (FI) and body weight (BW) were recorded. At d43, four birds from each pen within 10% of average bodyweight were selected for carcass yield and meat quality. Data were analyzed using one-way ANOVA model for a completely randomized design using the GLM procedure of SAS 9.4. Significant level was set at $P < 0.05$. During d 0-14 period, broilers fed a diet contained almond hulls at 2.5% showed a higher ($P = 0.025$) body weight gain compared to the control group. Supplementation of almond hulls quadratically increased ($P \leq 0.01$) broiler body weight gain and feed intake during d0-14 period with the peak dosage at 2.5%. Similarly, during the whole 42d grow-out period, supplementation of almond hulls quadratically ($P = 0.013$) increased broiler body weight gain. Meanwhile, graded almond hulls inclusion in broiler diet linearly ($P < 0.01$) increased the feed conversion ratio (FCR) during d 0-14, d 0-28 and d 0-42 period. In addition, broilers fed 2.5% of almond hull had the highest hot carcass, chilled carcass, wings and whole legs weight among all the treatments. All the treatments showed a similar white striping and woody breast scores. Supplementation of almond hulls at 5 and 7.5% showed a higher ($P < 0.01$) lightness value on breast fillet compared to the control group, whereas increasing the almond hull level in the diet linearly decreased ($P < 0.01$) pH and lightness values of breast fillet. However, there is no difference in lightness and pH of breast fillet between control and 2.5% supplementation of almond hulls. In conclusion, almond hull inclusion at 2.5% showed a positive effect on growth performance and carcass yield with a comparable breast fillet quality compared to a corn soybean meal control diet.

Key Words: Broiler; Carcass yield; Meat quality; Growth performance; Almond hull

244 Effect of dietary soy galactooligosaccharides on AMEn, nitrogen retention and ceca microbiome of broiler chickens. Kyle Brown^{1*}, Kyle Teague², Guillermo Tellez-Isaias², Jianmin Chai³, Simone Rasmussen¹, Alfred Blanch¹, Samuel Rochell², Jiangchao Zhao², ¹*Hamlet Protein A/S, Horsens, Denmark*, ²*University of Arkansas, Fayetteville, United States*, ³*Foshan University, Foshan, China*.

The objective was to investigate the effects of increasing levels of the soy galactooligosaccharides (GOS) raffinose and stachyose in feed on performance and gastrointestinal health including AMEn, nitrogen retention and ceca microbiome in young chickens in a randomized complete block design. Results of performance, intestinal morphology and permeability and cell immunology have been published previously. A 21-d trial was conducted with 400 d-old Cobb 500 male chickens placed in 50 battery cages (8 birds/cage; 0.04 m²/bird). Replicate cages were assigned to one of five treatments: a control diet devoid of the soy GOS based on soy protein isolate (SPI) or the same SPI diet with 0.9, 1.8, 2.7 or 3.6% added GOS (a mix of stachyose and raffinose, 4:1 ratio, as found in soybean meal). Birds had ad libitum access to feed and water. Feed intake and excreta output were measured from d12-14 and d18-21 and homogenous excreta samples were collected to determine nitrogen retention and AMEn. At d21, ceca digesta was collected for microbiome analysis. The V4 region of the bacterial 16S rRNA gene was sequenced on a MiSeq sequencer. Data were analyzed by one-way ANOVA. Means were compared by Tukey's multiple comparison test. Linear and quadratic regressions were used to investigate the dose-response of GOS in diets. The microbiome data were analyzed by using the QIIME2 software package. Linear discriminant analysis effect size was performed to identify the microbiome signature for differentiating groups. At d14, both linear ($p < 0.01$) and quadratic effects ($p < 0.01$) were observed for nitrogen retention, the maximum value being 70.20% at 1.24% GOS, based on the quadratic regression. At d21, nitrogen retention decreased linearly ($p < 0.001$) with increasing GOS level. AMEn increased according to a quadratic regression at d14 ($p < 0.03$) and d21 ($p < 0.001$), the maximum values being 4,073 kcal/kg DM at 1.61% GOS and 3,911 kcal/kg DM at 1.44% GOS, respectively. GOS supplementation significantly affected ceca microbiome. 1.8, 2.7 and 3.6% GOS significantly decreased alpha diversity including Shannon Index and the number of Observed Amplicon Sequence Variants (ASVs) compared to 0 and 0.9% GOS, and showed distinct cluster based on Bray-Curtis and Jaccard distances. *Ruminococcus* (ASV14, ASV27 and ASV28), *Coprococcus* (ASV22) and *Oscillospira* (ASV59) were increased with increasing GOS-levels, while *Lachnospiraceae* (ASV40), *Butyricicoccus pullicaecorum* (ASV127) were increased in the control group. *Blautia* (ASV80) had higher abundance in the control and

0.9% GOS groups. In conclusion, results from this trial indicate that soy GOS have dose-dependent effects on the nitrogen retention, AMEn and microbiome in broiler chickens from d0-21.

Key Words: AMEn; broilers; microbiome; nitrogen retention; Soy galactooligosaccharides

245 Effects of the nutritional density of the diet and whole wheat supplementation on growth performance and gizzard development of broilers from 0 to 40 days of age. H. Kadardar¹, L. Aguirre¹, G. Talegón¹, R. Lázaro¹, L. Cámara¹, Gonzalo Mateos^{1*}, ¹*Universidad Politécnica de Madrid, Madrid, Spain.*

The effects of the nutrient content of the diet and the extra supplementation with whole wheat (WW) on growth performance and proventriculus and gizzard development, were studied in two broiler strains from 0 to 40 d of age. The experimental design was completely randomized with 12 treatments organized as a $2 \times 2 \times 3$ factorial, with two broiler strains (A vs. B), two feeding programs (high vs. medium quality feeds), and three levels of WW inclusion (control vs. low vs. high). The feeding program consisted of 3 phases (0 to 14 d, 15 to 28 d, and 29 to 40 d of age) and all diets were fed in pellet form. In each phase, the medium quality diets had 100 kcal AMEn/kg less and 5% lower digestible essential amino acids content than the high quality diets. The control diets did not add any supplemental WW, whereas, the experimental treatments where supplemented post-pelleting with 3% of WW from 0 to 14 d and 7.5% or 15% of WW, depending on treatment, from 15 to 40 d of age. Each treatment was replicated 6 times and the experimental unit was a pen with 16 chicks. Growth performance, energy intake (EI; kcal AMEn/d), and energy conversion ratio (ECR; kcal AMEn/g BW gain) were measured by feeding phase and cumulatively (0 to 40 d of age). Additionally, two birds per replicate were slaughtered at 40 d of age and the proventriculus and the gizzard were excised for further examination. Data were analyzed as a completely randomized design using the MIXED procedure of SAS, with broiler strain, nutritional density of the diet, WW supplementation as main effects as well as their interactions. Cumulatively, birds from the A strain ate more feed (125 vs. 118 g/d; $P < 0.001$) and grew faster (81.6 vs. 77.0 g/d; $P < 0.001$) than birds from the B strain but FCR was not affected. Birds fed the high quality diets were heavier (80.6 vs. 78.0 g/d; $P < 0.001$) and had better FCR (1.504 vs 1.569 g/g; $P < 0.001$) than birds fed the medium quality diets. Supplementation with WW reduced FI and BW gain of the birds and impaired FCR ($P < 0.001$). An interaction between bird strain and WW supplementation was detected from 29 to 40 d of age: WW supplementation impaired FCR in all the birds but the effects were more evident for strain A than for strain B ($P < 0.001$). Birds fed WW presented heavier gizzard (% BW) that had a lower pH than broilers fed the control diet ($P < 0.001$). In summary, growth performance was greater for broilers of the A strain

than for broilers of the B strain. An increase in feed quality increased BW and improved FCR but did not affect ECR. Supplementation of WW post-pelleting (on top of the original feeds) improved gizzard development at 40 d of age but reduced BW gain, independently of bird strain and type of diet.

Key Words: growth performance; gizzard development; nutritive density of the diet; whole wheat feeding

246 Modelling the impact of corn quality on broiler feed conversion ratio: a meta-analysis. Carrie Walk^{1*}, Levy Teixeira³, Jose Otavio Sorbara², ¹*DSM Nutritional Products, Heanor, United Kingdom*, ²*DSM Nutritional Products, Kaiseraugst, Switzerland*, ³*DSM Nutritional Products, Mairinque, Brazil.*

Variation in corn quality, due to genetic variety, growing, harvest, and processing conditions can influence the apparent metabolizable energy (AME) of corn and subsequently impact broiler feed conversion ratio (FCR). A meta-analysis was carried out to quantify the impact of corn quality, corn non-starch polysaccharides (NSP), and in vitro starch digestibility on broiler FCR. The analysis was carried out on data from 23 broiler trials conducted in North (58%) and South (42%) America between 2017 and 2019. In all trials, broilers were fed corn and soybean meal-based diets formulated to meet or exceed nutrient requirements. There were a total of 66 datapoints. All trials started on day of hatch and concluded between day 33 or 49, with the majority (73%) of the trials ending on day 42. Corn samples from all of the trials and feeding phases were collected and analyzed for total and insoluble NSP monosaccharides (including arabinose, xylose, and glucose), in vitro starch digestibility (including rapidly digestible starch, slowly digestible starch, resistant starch, and total starch) and corn quality parameters including protein solubility (Promatest) and vitreousness. Corn quality, starch digestibility, and NSP data, as well as broiler FCR were analyzed using step-wise regressions in the fit model platform of JMP Pro v. 15.1. A portion of the data (70%) was used to build the model and 30% of the data was used to validate the model. Correlations between factors were evaluated and removed or incorporated into the model as interaction terms. Non-significant model effects were removed, unless part of a significant interaction. The final model was selected based on R^2 (0.79), cross validation R^2 (0.66), and significance of the factors ($P < 0.05$). The term 'end phase day' was included in the model to account for the natural increase in FCR as the birds age ($P < 0.0001$). Corn quality parameters predicted to significantly negatively influence broiler FCR included: increasing vitreousness ($P = 0.01$), greater insoluble A:X ratio ($P = 0.0001$) and a higher content of slowly digestible starch ($P = 0.02$), whereas increasing protein solubility ($P = 0.006$) from 27 to 50 eq. mg albumin was predicted to improve broiler FCR, but only if resistant starch was less than 13 g/100g (interaction, $P = 0.03$). Inputting the results from a naïve sample of corns harvested

in 2020, broiler FCR was predicted to differ by approximately 12 points (1.57 vs. 1.45). These results highlight the significant impact corn quality parameters, such as vitreousness, insoluble A:X ratio and slowly digestible starch have on broiler FCR and relationships between protein solubility and resistant starch.

Key Words: corn; feed conversion ratio; protein solubility; non-starch polysaccharide; vitreousness

247 Prediction of the energy content of soybean meals for poultry based on their chemical composition. A practical approach. G. Fondevila¹, L. Aguirre¹, L. Cámara¹, A.F. de Juan¹, N. Corrales¹, Gonzalo Mateos^{1*}, ¹Universidad Politécnica de Madrid, Madrid, Spain.

Commercial batches of soybean meal (SBM) from Argentina, Brazil, and USA (n = 9 per origin) were collected at random from different feed mills in Europe. The AMEn values of the SBM were estimated from their wet chemical composition according to published prediction models (WPSA, 1989; Rostagno et al., 2017; CVB, 2021; FEDNA, 2021). In addition, the apparent ileal crude protein digestibility (dCP) and the AMEn of the samples were determined *in vivo* in 21 d-old broilers. Recently, a new prediction equation based on the WPSA (1989) model was developed at the Universidad Politécnica of Madrid to improve the accuracy of the estimated AMEn values of commercial SBM. In this approach, the original equation recommended by WPSA (1989) [$\text{AMEn (kcal/kg)} = 37.5 \times \text{crude protein} + 46.4 \times \text{ether extract} + 14.9 \times \text{nitrogen free extract}$] was modified based on published differences in the digestibility of the carbohydrates, protein, and ether extract fractions. The resulting UPM (2022) equation (88% DM basis) was $\text{AMEn (kcal/kg)} = 69.1 + 43.1 \times \text{crude protein} \times \text{dCP} + 55.7 \times \text{ether extract} + 37.3 \times (\text{starch} + \text{sucrose}) + 12.4 \times \text{oligosaccharides}$, where all components are presented in percentages, ether extract was analyzed after previous acid hydrolysis, and dCP values were those determined in the *in vivo* trial. The estimated AMEn values of the SBM, according to the origin of the beans, and the interactions between origin and the prediction equation used, were analyzed using the MIXED procedure (SAS Institute, 2018). In addition, the Pearson correlation (r) analyses was used to determine the correlation between *in vivo* dCP (86.5, 86.1, and 87.1% for SBM from Argentina, Brazil, and USA, respectively) and AMEn (2,307, 2,281, and 2,323 kcal/kg for SBM from Argentina, Brazil, and USA, respectively), and the estimated energy values using each individual prediction equation. The AMEn of the SBM varied according to the prediction equation used ($P < 0.001$) with the greatest value reported for the Rostagno et al. (2017) equation and the lowest for the CVB (2019) equation. Origin of the SBM did not affect the estimated AMEn content of the meals but the response varied according to the prediction model used ($P < 0.001$ for the interaction). In this respect, the *in vivo* dCP ($r = 0.428$; $P < 0.05$) and AMEn ($r = 0.411$; $P < 0.05$) of the SBM were

positively correlated with the energy value estimated using the UPM (2022) but not with any of the other equations. In summary, the variability of current prediction equations to estimate the AMEn of SBM have a wide variability and a poor correlation with *in vivo* dCP and AMEn values. Prediction equations for SBM should be based primarily on digestible protein followed by sucrose and ether extract contents.

Key Words: poultry; soybean meal; metabolizable energy; prediction equations

248 Chemical composition and nutritional value of raw soybeans from Brazil and USA arriving to European ports. L. Cámara¹, L. Aguirre¹, A.F. de Juan¹, J. Ben Mabrouk¹, H. Kadardar¹, G. Talegón¹, Gonzalo Mateos^{1*}, ¹Universidad Politécnica de Madrid, M, Spain.

The aim of this study was to compare the quality and chemical characteristics of raw beans exported to Europe from Brazil (BRA) and USA in 2021/2022. Representative samples (7 kg each) of raw beans from BRA (n = 12) and USA (n = 7) were collected at random from different processing plants in Europe by specialized personnel. The samples were classified in the lab into high quality (HQ) and damaged seeds (whole and splitted beans included) and foreign material. The damaged (3.2 vs. 6.3% for the USA and BRA) and the HQ (93.2 vs. 91.9% for USA and BRA beans) raw beans were analyzed for major constituents in the same lab by the same technician, according to official methods. Chemical composition (CP, EE, sucrose, oligosaccharides, CF, and NDF), protein quality indicators [urease activity (UA), PDI, KOH, trypsin inhibitors activity (TIA)], and oil quality traits (oleic acidity and peroxide index) were determined. Data were analyzed as a completely randomized design, with country of origin of the beans (USA vs. BRA) and bean quality (damaged vs. HQ) as main factors, using MIXED procedure of SAS. Independent of the country of the origin of the beans, damaged raw beans had more DM, CP, and less sucrose and stachyose than HQ beans ($P < 0.05$ to $P < 0.001$, depending on the trait). Protein quality indicators differed also between damaged and HQ beans with PDI ($P < 0.001$), KOH ($P < 0.001$), and TIA ($P < 0.05$) values being lower for the damaged beans than for the HQ beans. Damaged raw beans had higher peroxides index and oleic acidity than the HQ beans ($P < 0.001$). Country of origin of the beans did not affect CP of damaged beans (36.6 vs. 37.0%; $P > 0.10$). Damaged beans from USA had higher sugar and oligosaccharides content than BRA beans ($P < 0.05$). The quality of the CP and lipid fractions was also affected by the country of origin of the beans, with lower UA ($P < 0.05$), PDI ($P < 0.001$), TIA ($P < 0.01$), and peroxide index ($P < 0.01$) for the damaged beans from BRA than for the USA. Ether extract ($P = 0.001$), DM ($P = 0.082$), CP ($P = 0.093$), and NDF ($P = 0.088$) were lower for the HQ USA than for the HQ BRA beans. Also, USA HQ beans had more sucrose ($P < 0.05$) and stachyose ($P < 0.01$) and higher UA ($P <$

0.05), PDI ($P < 0.01$), and TIA ($P < 0.01$) values than BRA HQ beans, but no differences were found for KOH. It is concluded that USA raw beans have lower percentage of damaged grains than Brazilian raw beans. Irrespective of the origin, damaged beans had more CP ($N \times 6.25$) and less sucrose and oligosaccharides than HQ beans. CP and lipid fractions quality were reduced in the damaged beans.

Key Words: chemical composition; protein quality indicators; damaged bean; high quality raw bean; raw soybean origin

249 Chemical composition, protein quality, and protein digestibility and metabolizable energy content of commercial soybean meals from different origins in 21-day old broilers. L. Aguirre¹, L. Cámara¹, G. Fondevila¹, A.F. de Juan¹, J. Ben Mabrouk¹, Gonzalo Mateos^{1*}, ¹Universidad Politécnica de Madrid, Madrid, Spain.

We studied the chemical composition, protein quality, in vitro protein digestibility (IVPD), and in vivo apparent ileal protein digestibility (AIPD) and AMEn content, of 27 soybean meal (SBM) samples from Argentina (ARG), Brazil (BRA), and USA. The samples ($n = 9$ per country) were collected at random from European crushers and feed mills by specialized personnel, and analyzed for main nutrients (CP, amino acids, crude fiber, neutral detergent fiber, sucrose, oligosaccharides, and minerals) and protein quality indicators (urease activity, KOH solubility, protein dispersibility index, and trypsin inhibitor activity). The IVPD of the meals was determined using a two-step test that simulated the digestive processes occurring in the gastrointestinal tract of the chick. In addition, the AIPD and the AMEn content of the SBM were determined in 21 d-old broilers. Broilers were fed a commercial crumble diet based on cereals and SBM from hatch to 17 d of age and then, they received their corresponding experimental diet ad libitum for 4 d. The experimental diets consisted of 53% of a common N-free diet (44.0% dextrose, 3.5% soybean oil, 3.5% vitamins and minerals, and 2.0% lignocellulose) and 47% of the SBM tested, as the unique source of N. All data were analyzed as a completely randomized design using the MIXED procedure of SAS with the origin of the SBM as main effect. Additionally, for the in vivo data, SBM sample within each origin was used as a nested effect where each of the 27 samples was replicated 6 times and the experimental unit was a cage with 8 broilers. On 88% DM bases, SBM from BRA had more CP (46.5, 45.4, and 45.5%; $P < 0.05$) and less sucrose (5.21, 6.28, and 6.47%; $P < 0.001$) and stachyose (4.20, 4.66, and 4.78%; $P < 0.05$) than SBM from USA and ARG. Protein quality indicators values were higher for the USA meals than for the BRA and ARG meals ($P < 0.05$). At 3 h post-incubation, IVPD values tended to be higher for the USA meals than for the ARG and BRA meals ($P = 0.078$). At 21 d of age, AIPD of the SBM tended to be higher (87.1, 86.5, and 86.1%, respectively; $P = 0.063$) for the USA meals than for the ARG and BRA meals. Also, the AMEn of the SBM was 2,323, 2,307, and 2,281 kcal/kg

for the USA, ARG, and BRA meals, respectively, differences that were not significant in spite of the higher CP content of the BRA meals. In summary, the chemical composition, protein quality indicators, and nutrient quality of the SBM varied with the origin of the beans. Feed mill managers and nutritionists should take into account the composition and origin of the beans, to create a more accurate matrix of nutritional values for commercial SBM.

Key Words: apparent metabolizable energy; broiler; soybean meal origin; in vitro N digestibility; ileal protein digestibility

250 Effects of dietary feed additives on egg quality, cholesterol and fatty acid profile of laying hens. Farhana Sharmin^{1*}, Sazedul Sarker¹, Md Ataul Goni Rabbani¹, ¹Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh.

Eggs are highly favored as an excellent source of nutrients than can be designed through dietary manipulation of specific nutrients. The fatty acid profile and cholesterol of eggs can be changed by the alteration in the diet in poultry egg. The objective of the study was to evaluate the feeding effect of *Moringa oleifera* leaf (MOL) meal, *Linum usitatissimum* (Flaxseed) and *Trigonella foenum-graecum* (Fenugreek) on egg quality, fatty acid profiles and cholesterol content of egg yolk. A total of 168 Fayoumi laying chickens at age 33 weeks were selected for this study, and the experiment continued until they were aged 49 weeks. Birds were distributed according to a completely randomized design of experiment. Seven dietary treatment groups were produced from the basal feed as follow T₁-Control Diet; T₂-Flaxseed 0.5% + MOL 1%; T₃-Flaxseed 1% + MOL 1%; T₄-Flaxseed 1.5% + MOL 1%; T₅-Fenugreek 0.5% + MOL 1%; T₆-Fenugreek 1% + MOL 1%; T₇-Fenugreek 1.5% + MOL 1%. After completed the experiments (4 months) egg quality such as egg weight, shape index, shell thickness, shell weight (g), albumen index, yolk index and yolk color were measured. In addition, cholesterol and fatty acid profile were determination by GC. Statistical analysis was conducted using one-way ANOVA with the SPSS statistical package (Version 16.0, NY, USA). Feeding flaxseed and fenugreek with MOL (1%) meal up 0.5 to 1.5% had no adverse effects on the external or internal qualities of eggs and improved the egg weight in additives group (51.58 to 54.72g) compared to the control (T₁) group (48.07g). Higher amounts of total omega-3 fatty acid ($P < .05$) were found in the eggs of T₂ and T₅ group with values of 1.86 and 1.30% respectively, and total PUFA content was also higher ($P < .05$) in the T₂ and T₅ groups (17.44 and 18.31 %, respectively) compared to the control (12.33%). The results obtained in this study revealed that the addition of different levels of feed additives in the hens' diets had no effect on production performance in terms of egg nutritional composition and external quality parameters of egg. Fatty acid composition, especially omega-3 fatty acid content,

was improved, and egg cholesterol concentration was numerically reduced at the lower levels i.e., 0.5% of flaxseed and fenugreek with MOL 1% added to diet. Therefore, it can be concluded that diets up to 0.5% dietary flaxseed and fenugreek with MOL 1% could be used in layers' diets. However, further follow-up research is required to know the mechanisms of various pathways of reducing cholesterol and enrichment of omega-3 fatty acids content in the egg yolk.

Key Words: Fayoumi laying hen; designer egg; Flaxseed; Fenugreek; Fatty acid profiles

251 Dietary supplemental with fermented soybean meal improves the growth performance, intestinal health and immune performance of laying hens during the brooding period. Zhu-jin Lu^{1*}, Xiu-qi Wang¹, Chun-qi Gao¹, ¹*South China Agricultural University, Guangzhou, China.*

The banning of antibiotics in feed has been a global trend. Fermented soybean meal (FSM) is expected to be a suitable substitute for antibiotics because of its benefits to improve intestinal health levels and immune performance. This study aimed to investigate the effects of FSM supplementation on the growth performance, intestinal health, and immune performance of laying hens during the brooding period. Four hundred eighty healthy 1-day-old laying hens were divided randomly into 4 groups (6 replicates/group, 20 hens/replicate) and were fed with different experimental diets from the day 1 (D1) to D56: (1) Control diet (CON); (2) Chlortetracycline (CTC; A diet supplemented with 0.5g chlortetracycline per 1kg of feed); (3) 4% fermented soybean meal (4% FSM; A diet containing 4% fermented soybean meal); (4) 8% fermented soybean meal (8% FSM; A diet containing 8% fermented soybean meal). Feed consumption was determined and average daily gain (ADG), average daily feed intake (ADFI), and FCR were calculated. After the trial ended, we collected the hens' samples of serum, jejunal tissue, and immune organ, which were detected and analyzed. Simultaneously, immunofluorescence and Western blotting were used to detect the critical protein expression of Wnt/ β -catenin signaling and evaluate intestinal development. The results showed that the ADFI and ADG of hens were significantly enhanced by adding 4% or 8% FSM, and the same effect as the CTC group compared with the CON group ($P < 0.05$). Meanwhile, the group of 4% FSM or 8% FSM improved the integrity of jejunal structure and barrier, reflected in increased villus height, enhanced protein expression of absorptive cell (Villin), tight junction proteins (Occludin and Claudin-1), proliferative cell marker, progenitor cell marker (SOX9), and stem cell marker (Lgr5), which has the same effect with the CTC group. Furthermore, CTC or 4% FSM treatment group resulted in a remarkable increase in

Wnt/ β -catenin signaling proteins (including β -catenin, TCF4, c-Myc, and Cyclin-D1) compared with the CON group ($P < 0.05$). Moreover, the results of immune organs showed that compared with the CON group, 4% FSM and 8% FSM could significantly improve the spleen index and thymus index of laying hens during the brooding period ($P < 0.05$). In addition, the IgA, IgM, and IgG levels in the serum of 4% FSM or 8% FSM groups were significantly increased than in the CON group ($P < 0.05$). Overall, dietary supplementation of 4% fermented soybean meal in the diet improves growth performance, intestinal health, and immune performance of laying hens during the brooding period.

Key Words: growth performance; intestinal health; Laying hens; fermented soybean meal; immune performance

252 Effects of high fat diet on growth performance, serum parameters and hepatic lipid metabolism in Pekin ducks. Guan Chen^{1,2} Keying Zhang^{1,2} Gang Tian^{1,2} Ping Bai^{1,2} XueMei Ding^{1,2} Jianping Wang^{1,2} Li Lv^{1,2} Yue Xuan^{1,2} Qiufeng Zeng^{1*2}, ¹*Sichuan Agricultural University, Chengdu, Sichuan, China,* ²*Key Laboratory for Animal Disease-Resistance Nutrition of, Ministry of Education, Ministry of Agriculture and Rural Affairs, Sichuan Province, Chengdu, China.*

This study aimed to investigate the effect of high fat diet on growth performance, serum and hepatic lipid metabolism in Pekin ducks from 10 to 40 d of age. Two hundred and forty healthy male Pekin ducks (10-day-old, 470.53 ± 0.57 g) were randomly divided into three corn-soybean meal-based diet groups (8 replicates per cage of 10 ducks): a normal diet (ND, 3% fat), a high fat diet (HFD, 9% fat), and a pair-fed diet (PFD, gave the ND in an amount equal to that consumed on the HFD to eliminate the effects of feed intake). Data were analyzed using a one-way variance and significance different at $P < 0.05$ evaluated using student's t-test (SAS 9.2; SAS Institute Inc., Cary, NC). Results showed as follows: Compared to ND, HFD significantly decreased the feed intake and FCR, along with serum triglyceride (TG) and free fatty acid (FFA) level, total antioxidant capacity (T-AOC) and thioredoxin oxidoreductase activities, as well as liver weight, liver index, area of lipid droplets (Oil Red O staining), malate dehydrogenase (MDH) activities. When compared with PFD, HFD significantly decreased FCR, serum total cholesterol, hepatic carnitine palmitoyltransferase 1, MDH, glucose 6 phosphate, and lipoprotein lipase activities as well as the content of T-AOC of ducks. In conclusion, HFD increased feed efficiency by reducing feed intake and improving serum and hepatic lipid metabolism; but HFD presented to decrease the antioxidant capacity of serum and liver of ducks.

Key Words: feed efficiency; lipid metabolism; antioxidant capacity; ducks; High fat diet

Extension and Instruction

253 Dirty birds: Employment of critical thinking to address misconception seeded by documentaries opposed to conventional poultry production. Drew Benson^{1*}, ¹*University of Georgia, Athens, Georgia, United States.*

It is important that Poultry science graduates are prepared to address both criticisms and propaganda that are aimed at vilifying poultry production. Agricultural education needs to prepare our students with critical thinking exercises and assessments for dealing with the combination of increased consumer detachment from agriculture and an increased presence of propaganda critical of industrial agricultural production. With the increase in online media content focusing on food production, consumers are increasingly able to obtain information from documentaries focused on agricultural production, including the poultry industry. One such mini documentary, “Dirty Birds: A Story of Chickens in America,” criticizes conventional broiler production and compares it with pastured broilers. Each Fall and Spring semester, from Fall 2018 to Fall 2021, students in Introduction to Poultry Science (POUL 2020) were assigned to watch the documentary and fill out a survey on the first week of class. At the conclusion of the course, which primarily covers poultry production, the students retake the same survey and also respond to their original answers to open ended questions. The survey, which was a mixture of open-ended questions and five-point Likert scale responses, sought to determine the perceptions of the students of the poultry industry, as well as their critical thinking that led to their decision making, following viewing of the documentary before and after completion of the course. The open-ended questions were analyzed as a performative assessment for critical thinking across four central facets developed by The International Performance Assessment of Learning (iPAL). Quantitative data sets were analyzed using SPSS and one-way ANOVA was used to compare pre- and post- survey responses. The results indicate that students (n=192) increased their knowledge and understanding of the poultry industry and had a more favorable opinion concerning the poultry industry (p<0.05) following completion of the course. In addition, the open-ended response questions scored higher, based on iPAL scoring, for critical thinking especially in increased use of evidence and knowledge in support of their opinion. The students also acknowledged the value of the metacognition that this exercise provided concerning the importance of making informed opinions and how they can properly address misconceptions. This type of exercise provides students with the skills needed to appropriately respond to ubiquitous misconceptions seeded by the increasing presence of media that is critical of agricultural production.

Key Words: teaching; critical thinking; Assessment; misconceptions

254 Bringing the farm to the student: a virtual turkey scenario to educate undergraduate students about turkey welfare and production. Marisa Erasmus^{1*}, ¹*Purdue University, West Lafayette, Indiana, United States.*

Many students entering into undergraduate programs are unfamiliar with animal agriculture. Courses are one way for students to learn about poultry production practices; however, it is not always possible to bring students to farms due to biosecurity concerns, the distance of farms to universities or the costs associated with transporting students to farms. With the various technologies that are available, it is possible to educate students about poultry welfare and production practices using virtual scenarios. The use of scenarios is not new; for example, the American Veterinary Medical Association (AVMA) uses scenarios as part of the Animal Welfare Judging and Assessment Contest every year. However, the use of a scenario for educating students about turkey production has not previously been examined. This study examined undergraduate students’ perceptions and content knowledge before and after completing a virtual turkey welfare scenario. The virtual scenario was developed in conjunction with the Purdue University Envision Center for Data Perceptualization and featured two fictitious turkey farms. Although the farms were fictitious, details about each farm, such as hatchery procedures, animal-based welfare indicators and processing procedures, were designed to be reflective of current turkey farming practices and scientific literature. Data were collected from students in 2019 (26 students), 2020 (35 students) and 2021 (18 students; n=79 students total). Students were required to complete a written report and two quizzes related to the scenario as part of a graded assignment in the class. To access the scenario, students had to complete a quiz (pre-quiz). Then, students were asked to complete a post-quiz after all students had read through the scenario and completed their written report. As part of the quizzes, students were asked to rate their level of interest in, and knowledge of turkey production on a scale of 1 (not interested) to 7 (very interested). Self-reported interest in turkey production increased in 44.30% (pre: 2.97 ± 0.17 vs. post: 3.37 ± 0.19 ; mean \pm SE score), and knowledge of turkey production increased in 89.87% (pre: 2.26 ± 0.13 vs. post: 4.32 ± 0.11 score) of students after completing the assignment. Students’ performance on general content questions also improved after completing the assignment, with the percentage of students answering correctly increasing by 65.82%-67.92% after completing the assignment. In summary, the use of a virtual scenario is beneficial in improving undergraduate students’ interest in, and knowledge of turkey production and may benefit students in areas where in person farm visits are not possible.

Key Words: interest; undergraduate education; knowledge; turkey production; virtual farm scenario

255 Multi-state poultry feeding and nutrition conference: A 39 year look back. Ken Koelkebeck^{1*}, Carl Parsons¹, Jason Emmert¹, Anthony Pescatore², Sunday Adedokun², Mike Lilburn³, Darrin Karcher⁴, Williams, Zachary⁵, ¹*University of Illinois, Urbana, Illinois, United States*, ²*University of Kentucky, Lexington, Kentucky, United States*, ³*The Ohio State University, Wooster, Ohio, United States*, ⁴*Purdue University, West Lafayette, Indiana, United States*, ⁵*Michigan State University, East Lansing, Michigan, United States*.

Poultry extension education is important to today's poultry industry because it helps maintain visibility with clientele and provide current information. The poultry extension specialists at Purdue University, Michigan State University, The Ohio State University, University of Illinois, and University of Kentucky formed the Multi-State Poultry Extension group 39 years ago. Several extension educational programs have been conducted over this period of time, however, the longest running program developed has been the Multi-State Poultry Feeding and Nutrition Conference. The first conference was held in 1984 in Indianapolis, Indiana. It has been conducted every year except in 2020, when it was cancelled due to COVID-19 concerns. The aim of this meeting has been to provide practical and technical information concerning the proper feeding and nutrition of poultry to interested clientele. A key objective of this meeting has been to be self-sustaining due to the financial support and cooperation of poultry nutrition companies. The goals of this meeting have been to: 1) provide high quality educational information, 2) provide information for area poultry producers, 3) attract outstanding outside speakers, and 4) secure extramural funding. Funding for this meeting has come from poultry nutrition companies and revenue has been generated from meeting registrations. In addition, several major poultry nutrition companies have solely sponsored receptions and meals functions. This Multi-State program has been successful because of the work of the Purdue University Division of Conferences. The conference coordinator's office has been responsible for sending out the program, assembling proceedings, taking registrations, and taking care of many other specific tasks. In securing speakers for this meeting, we have had speakers from industry as well as academia. Topics have been practical in nature. Every year, we have had a sponsoring company put on a technical symposium to go along with the multi-state program. This joint relationship has worked out well over the years. An advantage to conducting this conference is that we can reach a larger audience than if we were to conduct this meeting individually in our own states. By doing this, we can address poultry nutrition topics and problems that are common to everyone because these poultry nutrition issues are regional. In summary, decreasing human and monetary resources for poultry extension programs will require more regional efforts. Programs such as this one can work if they are developed over a reasonable period of time. Thus, this multi-state poultry extension programming effort, should

serve as a model for other commodity and livestock extension groups.

Key Words: poultry nutrition; poultry feeding; multi-state programs; conferences; extension educational programming

256 An online poultry health and medicine course for non-poultry veterinarians. Anthony Pescatore^{1*}, Jacqueline Jacob¹, Roberta Dwyer¹, David Frame², Richard Fulton³, Mark Eclov¹, ¹*University of Kentucky, Lexington, Kentucky, United States*, ²*Utah State University, Spanish Fork, Utah, United States*, ³*Michigan State University, East Lansing, Michigan, United States*.

The number of small and backyard flocks continue to grow. Unfortunately, there is a shortage of veterinarians that will accept small poultry flock owners as clients. Prior experience with conducting in person training programs for veterinarians have shown that there is interest in learning about poultry health, however, the small veterinary practices with one or two veterinarians cannot afford to close the office to attend in person trainings. An online course has been developed that will allow veterinarians to obtain knowledge on poultry health and earn continuing education credits (CEUs). The course consists of 22 modules that cover avian anatomy and physiology, infectious diseases, internal and external parasites, necropsies and disease prevention and treatment. The course has eight hours of instruction with each module a separate unit with questions after each module. All modules were reviewed by independent poultry veterinarians. The veterinarians enrolled in the course have two years to complete all modules in order to get their CEUs. The course has received the American Association of Veterinary State Boards' Registry of Approved Continuing Education (RACE) approval for eight hours of CEUs and there is no credit for partially completed courses. Veterinarians that have completed the course may enroll in a social network (discussion group) for the exchange of information and experiences. The ultimate goal is to increase the number of veterinarians willing to serve the small poultry flock owners. Funding for this project was provided by USDA NIFA (Federal grant 2020-70024-33090).

Key Words: Online; Veterinarians

257 Cooperative participation and technical efficiency of poultry-egg farmers in Ogun State, Nigeria. Olubunmi Balogun¹, Daniel Babalola¹, Martha Olumide^{1*}, Adebimpe Adefelu¹, ¹*Babcock University, Ilishan Remo, Ogun State, Nigeria, Ilishan Remo, Nigeria*.

The inestimable economic potential of poultry egg production cannot be over-emphasized. It also plays a key role in addressing the problem of nutritional (protein) deficiencies, particularly among the resource-poor rural households in Nigeria. It is evident that the low productivity experienced in poultry production in the country is due to the production approach adopted by farmers. This study,

therefore, examined the cooperative participation and technical efficiency of poultry egg farmers in Ogun State, Nigeria. A multistage sampling technique was adopted to select poultry (egg) farmers for this study. Data were collected from 148 poultry egg farmers with the aid of a structured questionnaire. The data were analyzed using descriptive statistics, budgetary analysis, and the Cobb-Douglas frontier model. Results indicated that the mean age of the poultry egg farmers was 47.3 ± 11.1 years. The majority of the farmers (95.9%) were educated at the tertiary level and 63.5% of the farmers belonged to a cooperative society. The mean profit of the cooperative and non-cooperative poultry farmers was N11,485,600.12 and N5,888,311.04 indicating that poultry farmers in

cooperative had a higher profit than non-members. The results indicate that the number of feeds and supplements, drugs and medication, the quantity of water, number of cages, day-old chick, fuel/diesel, hired person-day labor, and family labor employed were the variables that significantly explained the technical efficiency in the poultry farms. The study recommends that poultry farmers should consider social network membership as an avenue to acquire management skills to make them efficient managers of resources.

Key Words: Feed; Poultry egg production; Social network; Technical efficiency

Processing and Products

258 Effects of on-farm poultry processing on defeathering. Ryan Giometti^{1*}, Alex Samoylov¹, Aklilu Georges¹, Wayne Daley¹, ¹*Georgia Tech, Atlanta, Georgia, United States.*

The humane treatment of broilers during processing is a priority for the poultry industry. Pre-slaughter routines such as broiler catching and transport are the most stressful stages of the broiler production process and are known to have significant physiological and behavioral effects. As part of these routines, broilers are manually captured, crated, and loaded onto trailers for transport from the grow-out farm to the processing facility. During this period, they may experience heat and environmental stress, bruising, and injury. These welfare issues are endemic to the current process and are not easily addressed through incremental innovation. To eliminate many bird welfare and well-being issues and increase processing efficiencies, the On-Farm Processing and Transport (FPaT) system is proposed. The envisioned mobile FPaT system modifies the process by stunning, killing, and shackling the broilers at the farm, thus minimizing live handling and eliminating live transport. A separate unit then transports the shackled carcasses to a poultry processing plant thus delaying processing. For an initial assessment of the operability of the FPaT system, teams from the Georgia Tech Research Institute, the University of Georgia, and the USDA processed broilers using a proof-of-concept shackle transport system and tested picking efficiency for delayed processed carcasses at the UGA Poultry Experimental Farm and Processing Facility. Four groups of 20 carcasses each, including one control and three treatment groups, were processed postmortem in two replicates. The control group was processed traditionally through water-bath electrical stunning, scalding, and defeathering. The three treatment groups were water-bath stunned, shackled, and held for 2, 4, and 6 hours before scalding and defeathering. After defeathering step, all carcasses were visually examined and scored on the scale from 1 to 3 for defeathering quality. A lower score corresponds to a lower quantity of remaining feathers. Mean feather scores recorded for four groups of carcasses were: 1.05 for the control group and 1.52, 1.45, and 1.57 for the 2, 4, and 6 hours groups respectively. One-way ANOVA test showed a statistical difference between the groups with a p-value of 0.0001. Tukey's honest significance test described the differences between each group's feather score mean. While it was found that the treatment groups possessed statistically significantly higher feather score means than the control group, no statistically significant differences were found among the treatment groups. Furthermore, the higher feather retention by the treatment groups was concentrated near specific locations such as the wingtips and tail.

Key Words: Poultry processing; Defeathering; Poultry shackle transport; Poultry welfare and well-being; Poultry feather retention

259 Using virtual reality to enable human-robot systems for food processing applications. Colin Usher^{1*}, ¹*Georgia Tech Research Institute, Atlanta, Georgia, United States.*

Robotics are ubiquitous in manufacturing. However, in food processing and natural products, robots struggles to find a foothold outside of relatively simple picking, placing, and packaging applications. While solutions exist for manipulating and physically handling natural products, it is usually the accuracy of the sensing and decision making algorithms that fall short. Given the high throughput nature of food processing systems, coupled with the typical 90-95% accuracy of highly tuned automated sensing systems, robots are just not as good as humans at performing certain tasks. We present a novel approach using virtual reality to allow a human to act as the decision maker for a robot system, closing this accuracy gap and enabling applications currently not possible given today's technology. In addition, we will present a concept where humans performing these tasks will teach the robot systems how to perform the task autonomously. In modern poultry processing, deboned product is a major staple. On both manual and automated deboning lines, product is still manually loaded onto cones. This task requires a person to manually load a chicken onto a cone at the rate of one every two to three seconds. We will demonstrate an approach that allows a robot to load the chickens onto a cone via a human providing the pose of the chicken to the robot via a virtual reality system. Live data of the scene is captured with 3D sensors, and sent to a virtual environment where a user can tell the robot where the chicken to load is physically located. The robot then picks and places the chicken onto the processing cone autonomously. This technology allows a person to remotely operate the robot on a processing line. This technology has far-reaching potential. In addition to enabling remote work possibilities, it also opens opportunity for disabled people to work in environments they would not otherwise be able to. A person who would no longer be loading chickens would instead be commanding a robot. If critical mass could be achieved with such applications, a gig-economy could be created where people with consumer grade virtual reality systems could log into a website and work on a processing line on-demand.

Key Words: virtual reality; robot; collaboration; corobot; cobot

260 Immersion chiller total dissolved solids effects on peracetic acid decay rates. Daniel Sabo^{1*}, Stephanie Richter¹, ¹*Georgia Tech Research Institute, Atlanta, Georgia, United States.*

The poultry industry relies on peracetic acid (PAA) as an antimicrobial agent to ensure food safety and control microbial contamination. The importance of PAA highlights the need to clearly understand PAA decay rates within immersion chillers and factors attributing to decay. Evidence shows PAA decays rapidly with high organic carbon loading. Organics are common in the chiller and appear in the form of total suspended solids (TSS), total dissolved solids (TDS), fats, oils, and greases (FOGs). Previously, we have shown the effects TSS and TDS have on PAA decay. Building upon previous study results¹, TDS components were individually tested for their effects on PAA stability. All tests started with an initial PAA concentration of 150 ppm. PAA was added to test water and mixed for sixty seconds before concentration was measured ($t=1$). PAA concentration was measured at five time points (1, 5, 15, 30, and 60 minutes) at a pH of 9.0 using two colorimetric adsorption device kits. All half-lives were calculated using an exponential decay formula. Standard Excel formulas were used to calculate averages and standard deviations. PAA in potable water (pH 9.0) acted as a control group for decay rate with a half-life of 236 minutes. Three forms of proteins, bovine serum albumin (BSA), collagen, and beta globulin at 2000 ppm, were tested first. Half-lives were calculated for each protein at 228, 205, 224 minutes respectively. Next, a combination of magnesium, potassium, sodium and calcium cations at 20, 160, 60, and 35 ppm respectively was tested and was found to cause PAA to have a half-life of 177 minutes. Next, PAA mixed with poultry blood (200 ppm) was tested and found to half-life of 176 minutes. A third study looked at the combined effects of cations and protein (BSA) on the decay rate of PAA. It was found that cations (magnesium, potassium, sodium and calcium cations at 20, 160, 60, and 35 ppm respectively) with BSA at 2000 ppm had a half-life of 71 minutes. A fourth study investigated the effects of cations combined with blood. The half-life of PAA under this condition was calculated to be 59 minutes. These results indicate that TDS components alone have a small effect on PAA stability. Once these components are combined, there is a large effect on PAA stability, with blood combined with cations having the largest impact.

Key Words: peracetic acid; PAA; immersion chilling; half-life

261 The effects of genotype and sex on carcass characteristics and meat quality in fast- and slow-growing dual-purpose chickens. Jaroslav Valenta^{1*}, Darina

Chodova¹, Eva Tumova¹, ¹*Czech University of Life Sciences Prague, Prague, Czechia.*

The aim of this study was to evaluate the effects of genotype and sex on select carcass characteristics and meat quality of fast- (Ross 308) and slow-growing (ISA Dual) chickens. A total of 1,320 birds (sex ratio 1:1) were placed into 8 indoor littered floor pens (2 genotypes x 4 replications). At an average live weight of 2 kg, randomly selected 20 males and 20 females of each genotype were slaughtered, eviscerated carcasses were chilled for 24 h at 4 °C, evaluated for carcass traits and manually deboned to calculate parts yield and analyze breast meat quality. The data was analyzed by two-way ANOVA using SAS (9.4), including the interaction of genotype and sex (GLM procedure). Irrespective of sex, Ross 308 reached 2 kg live weight at 35 d compared to 70 d for ISA Dual. Carcass traits were predominantly affected by genotype. However, the effects of genotype and sex were observed in breast meat ($P=0.027$) and abdominal fat ($P<0.001$) percentages. Irrespective of sex, Ross 308 chickens had higher breast meat yield than ISA Dual chickens whereas the later had higher abdominal fat percentage. Breast meat quality analysis indicated that Ross 308 had higher ($P<0.001$ for all) pH, cooking loss, and lower L^* values than ISA Dual. The effects of genotype and sex affected the a^* and b^* color values as well as texture parameters. ISA Dual males and Ross 308 males had the highest and the lowest a^* value, respectively ($P=0.002$) while females in both genotypes with intermediate a^* values did not differ from each other. The highest b^* value was in ISA Dual females, but for ISA Dual males and both sexes of Ross 308 did not differ ($P=0.006$). The ISA Dual, specifically females had the maximum shear force ($P=0.003$) than for Ross 308 (males or females). The genotype had stronger effects on chemical parameters ($P<0.001$ for all). The Ross 308 had lower contents of dry matter and crude protein, while cholesterol and ash contents had higher than ISA Dual. The effects of genotype and sex were found in intramuscular fat (IMF) content ($P<0.001$). The values were higher for Ross 308 than for ISA Dual. There was no difference in values between sexes for ISA Dual, but was higher for Ross 308 males than females. Results indicate predominant differences in evaluated parameters were due to genotype rather than to sex. The Ross 308 chickens had higher carcass yield and more tender breast meat, however, from nutritional point of view, the meat of ISA Dual had higher protein and lower IMF contents.

Key Words: carcass yield; meat quality; sex; genotype; dual-purpose

Metabolism and Nutrition, Amino Acids

262 Quantification of secretory IgA and crude mucin excretion and their contributions to endogenous amino acid losses in birds fasted, fed a nitrogen-free diet, or fed diets containing various highly digestible protein sources in precision-fed roosters. Benjamin Parsons^{1*}, Rebekah Drysdale², Jessica Cvengros², Pam Utterback², Samuel Rochell¹, Carl Parsons², Jason Emmert², ¹*University of Arkansas, Fayetteville, Arkansas, United States*, ²*University of Illinois, Urbana, Illinois, United States*.

The objective of this study was to quantify the total amount of secretory immunoglobulin A (sIgA) and mucin excreted in roosters fed diets containing highly digestible protein sources and to determine their proportional contributions to total endogenous amino acid (AA) losses. A series of precision-fed rooster assays with 24 h excreta collections were conducted using conventional White Leghorn roosters (4 to 8 roosters per treatment). In Experiment 1, roosters were fasted or precision-fed (crop intubation) 30 g of a nitrogen-free (NF) or semi-purified diet containing 10% casein. Roosters in Experiment 2 were fed 30 g of a NF or semi-purified diet containing either casein, whole egg, egg white, soy protein isolate, chicken breast meat, spray-dried animal plasma (SDAP), or a crystalline AA mixture providing the same AA as casein. A 4 × 4 Latin square design was used in Experiment 3, where roosters were fed 30 g of a NF or semi-purified diet containing either casein, whole egg, or crystalline AA to evaluate both diet and individual bird effect. The IgA and crude mucin concentrations were determined by ELISA and precipitation, respectively. Data from Experiments 1 and 2 were analyzed using a one-way ANOVA for a completely randomized design, whereas data from Experiment 3 were subjected to a one-way ANOVA with diet as a main effect and rooster as a random effect; differences were considered significant at $P < 0.05$. In Experiment 1, there were no differences ($P > 0.05$) in total mucin excretion among treatments; however, total IgA excretion was lower ($P < 0.05$) for fasted birds (2.65 mg), intermediate for birds fed a NF diet (6.69 mg), and highest for birds fed a casein diet (12.48 mg). Casein resulted in greater endogenous AA contributions from IgA, and endogenous AA losses (proportion of the total) from IgA were similar to that from mucin. In Experiment 2, mean AA digestibility for all protein sources was high (96-100%). No differences ($P > 0.05$) were observed for IgA excretion; however, casein, soy protein isolate, and SDAP increased mucin excretion compared with NF, whole egg, egg white, and chicken breast meat. Similar to Experiment 1, IgA contribution to endogenous AA was similar to that from mucin in Experiment 2. In Experiment 3, the total IgA and mucin excretion did not differ ($P > 0.05$) among treatments; however, IgA excretion differed greatly among individual roosters (7-27 mg; $P < 0.05$). In conclusion, fasting resulted in low IgA excretion, whereas the effects of highly

digestible dietary proteins on IgA and mucin excretion were inconsistent. Further, roosters were found to excrete a substantial amount of IgA, and the amount of endogenous amino acid losses from secretory IgA was similar to that from mucin.

Key Words: precision-fed; mucin; secretory IgA; endogenous amino acid; digestibility

263 Effects of different ratios of branched-chain amino acids on growth performance and body composition of broilers. Amit Singh^{1*}, Deependra Paneru¹, Jinquan Wang¹, Woo Kim¹, ¹*University of Georgia, Athens, Georgia, United States*.

This study aimed to investigate whether the higher inclusion of branched-chain amino acids (BCAA) than the standard requirement would improve or deteriorate the growth performance of broilers. We evaluated the effects of different ratios and levels of BCAA including leucine (Leu), isoleucine (Ile), and valine (Val) on production parameters and body composition of broilers. A total of 600 one-day-old male broiler chicks (Cobb 500) were randomly allocated to 6 dietary treatments ($n=10$ cages) and were offered corn-soybean meal-based mash feed in 2 phases (starter, d 0-10 and grower, d 11-21) based on Cobb broiler nutrient specification. The treatments were: i) Control, with standard BCAA levels (Leu: 1.85% and 1.71%; Ile 0.89% and 0.80%; Val 1.0% and 0.91%, in starter and grower phases, respectively) ii) High crude protein (CP) control (HCP control; contained CP equal to the high branched-chain amino acid treatment (HBCAA)) iii) High Leu (HLeu; contained double amount (2×) of Leu than that in the Control), iv) High Ile (HIle; contained 2×Ile than that in the Control), v) High Val (HVal; contained 2×Val than that in the Control), and vi) High Leu + Ile + Val (HBCAA; contained 2×Leu, 2×Ile, and 2×Val compared with the Control). During the starter phase, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were different across the treatments ($P < 0.05$). The HCP control showed similar performance as the standard Control. The HBCAA treatment reduced ADG (20.9 g vs 23.4 g and 23.2 g) compared with both controls and decreased ADFI (25.3 g vs 27.7 g) compared with the Control ($P < 0.05$). During the total period, ADG and FCR had a trend ($P < 0.1$) across the treatments, whereas ADFI was not different ($P > 0.05$). On d 21, the HBCAA had a 20% less bone mineral content (BMC) compared with the Control (10.4 g vs 13 g; $P < 0.05$), whereas the other treatments had intermediate BMC. The bone surface area in HBCAA was also lesser compared with the Control (74.7 cm² vs 90.2 cm²; $P < 0.05$), whereas the total body bone mineral density (BMD) was not different across the treatments ($P > 0.05$). The lean mass in HBCAA was also lower compared with both controls (650 g vs 745.3 g and 755.6 g; $P < 0.05$). Overall, the results suggest that the

addition of all BCAA twice their standard inclusion would reduce the growth performance, muscle accretion, and bone mineral deposition of birds. The high CP does not affect the growth performance once the standard CP and AA requirements are met. The CP content of the grower diet (~20.5%) is typically lower than the starter (~22%), reducing the total BCAA content in the grower diet, and the older birds may become more capable of metabolizing this level of BCAA.

Key Words: broilers; branched-chain amino acids; leucine; valine; isoleucine

264 Interactive effects of dietary isoleucine and valine ratios to lysine in response to varying leucine to lysine ratios in heavy broilers. Jason Lee^{1*}, Alejandro Corzo², Marcelo Silva², Ruben Kriseldi², Roshan Adhikari³, Chance Williams⁴, ¹CJ Bio America, Goliad, Texas, United States, ²Aviagen, Huntsville, Alabama, United States, ³CJ Bio America, Downers Grove, Illinois, United States, ⁴Wayne Farms, Oakwood, Georgia, United States.

A study was conducted to understand the relationship among dietary branched-chain amino acids (BCAA) on live performance and carcass traits in Ross YPM × 708 male broilers during the final feeding stage of a large bird broiler program. A total of 864 d-old male chicks was randomly placed into 72-floor pens according to a 2ⁿ⁼³ full factorial in central composite design (CCD) with 18 runs (14 treatments+4 central points) and 4 replicates per run. A CCD design is constituted of a full, factorial, or fractional design. The points at the center of the experimental domain and the points outside this domain make it possible to estimate the curvature of the response surface. A common feeding program was adopted until 39 days. Subsequently, each pen received 1 of 15 dietary treatments from 39 to 55 days of age varying in digestible ratios of Ile:Lys (51 to 82), Val:Lys (62 to 88), and Leu:Lys (100 to 190) which were calculated according to their corresponded code matrix values (-, -1, 0, +1, +). Birds and feed were weighed at 39 and 55 d of age to determine body weight gain (BWG), feed intake, and feed conversion ratio (FCR). At 56 d of age, all birds were processed to determine carcass characteristics. Data were analyzed using JMP v.15 by adopting the response surface macro option. Body weight gain (1,607 g; *P* = 0.4388) and FCR (1.97; *P* = 0.002) from 39 to 54 d of age were optimized at the lowest Leu:Lys ratio (100) with Val and Ile:Lys ratios of 71 and 62 for BWG and 88 and 82 for FCR. Poorer BWG and FCR were observed as Leu:Lys ratio increased although increasing Val:Lys and Ile:Lys ratios alleviated the poor performance. Conversely, breast weight (1,342 g; *P* = 0.031) was maximized at the highest Leu:Lys ratio of 190%. Interestingly, live performance (BWG and FCR) was optimized at the lowest Leu:Lys ratios while breast meat weight was optimized at the highest Leu:Lys ratio indicating that BCAA influence body composition. These results revealed that optimum BCAA ratios to Lys may vary depending on the desired response

criteria and demonstrate the importance of maintaining proper Val and Ile ratios dependent on dietary Leu. In this experimental phase, live performance was optimized in diets with low Leu:Lys ratios; however, breast meat can be enhanced by increasing dietary Leu:Lys with the appropriate Val and Ile ratios.

Key Words: broiler; Valine; Isoleucine; Leucine; central composite design

265 Manipulation of digestive dynamics in reduced crude protein broilers diets by lipid encapsulation of non-bound amino acids. Peter Chrystal^{1*2}, Peter Selle¹, Sonia Liu¹, Shemil Macelline¹, Shiva Greenhalgh¹, Jean Fontaine³, Elizabeth Santin³, ¹Poultry Research Foundation, Hornsby, New South Wales, Australia, ²Complete Feed Solutions Ltd, Hornsby, New South Wales, Australia, ³Jefo Incorporated, St Hyacinth, Quebec, Canada.

The development of reduced crude protein (CP) broiler diets has occurred in tandem with increased availability of an array of supplemental non-bound amino acids (NBAA). Reducing dietary CP is accompanied by a decline in soybean meal and concomitant increases in cereal grain and NBAA content of feed. Furthermore, reducing dietary CP results in decreased nitrogen (N) and excreta output, resulting in improved litter. Thus, successful development of tangible reductions in dietary CP are accompanied by enhanced sustainability and improved welfare in chicken-meat production. In contrast to static digestibility coefficients of amino acids (AA) measured at the distal ileum in broiler chickens, protein digestive dynamics (DD) has been considered a four-tiered sequence of events. These events are initiated by digestion of CP within the gastrointestinal tract (GIT) lumen, followed by intestinal uptakes of oligopeptides and monomeric AA. The third phase transit of AA across the enterocytes of the GIT mucosa followed by the fourth post-enteral phase determines the metabolic fate of AA, resulting in either lean protein gain or, other numerous functional roles. The post-prandial fates of dietary AA may be attributed to differences between digestion kinetics of differing protein sources and, it is therefore necessary to consider the DD of protein-bound, oligopeptides and NBAA. The proportions of endogenous protein losses were estimated based on excreta AA profiles, utilising a published model. For example, a transition from 220 to 180 g/kg CP in wheat-based diets, supplemented with NBAA, increased the estimated proportion of microbial AA by a factor of 1.32 (from 29.2 to 38.6%) whilst the same transition in maize-based diets increased by a more modest factor of 1.10 (22.1 to 24.4%) at the distal ileum. Instructively, proportions of microbial AA in ileal digesta were negatively correlated to the apparent ileal digestibility coefficients of all 16 amino acids assessed over 8 dietary treatments (*P* < 0.001). Also, NBAA compete with glucose for absorption across the GIT enterocytes via Na⁺-dependent transporters and manipulating the rate at which NBAA are digested and

absorbed, holds promise for reducing possible “over-load” at the sites of absorption, within the proximal GIT. Thus, embedding a proportion of NBAA in a lipid matrix relies on endogenous bile salts and pancreatic lipase to emulsify and digest the lipid, thereby retarding the release of NBAA in the GIT. The use of “lipid encapsulation” technology of NBAA in tangibly reduced CP diets therefore warrants further investigation.

Key Words: broiler; encapsulation; digestive dynamics; reduced crude protein

266 Intestinal barrier function of broiler chickens fed different sources of methionine with or without a leaky gut challenge induced by synthetic glucocorticoid.

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Intestinal barrier function of broiler chickens is influenced by the concentration of dietary amino acids but there are limited data on the role of dietary methionine (Met). Additionally, the bioavailability and metabolic pathways associated with various Met sources are different. This study investigates the implications of different Met sources for intestinal permeability (InP) and genes influencing gut barrier functions in broiler chickens. Four grower dietary treatments were formulated including a no Met supplemented diet (deficient), and three diets supplemented with adequate Met supplied as DL-Met, L-Met, or Met hydroxy analogue free acid (MHA-FA) at 100% of the nutrient specification (AminoChick 2.0) with a relative bioavailability of 65% for the MHA-FA product and were fed to Ross 308 broiler chickens (n=96). On d 16 of age, from each treatment, 24 broilers were transferred to individual cages. On d 17, 19, and 23 of age, half of the birds in each dietary treatment were given a low dose of dexamethasone (DEX; 0.35 mg/kg) to induce gut barrier dysfunction. The remaining birds were sham injected with saline solution. On d 24, intestinal permeability (InP) for each bird was measured using fluorescein isothiocyanate dextran (FITC-d). The relative expression of genes encoding tight junction proteins (Claudin 1, 2 and 3) and regulating genes affecting the gut inflammation and integrity including nuclear factor- κ B (NF κ B) and glucagon-like peptide 2 (GLP2) were assessed by qPCR on jejunal tissues collected on d 24. Data were analyzed by two-way ANOVA to assess the effects of diet, DEX, and their interaction. Fisher's LSD test was used to separate means ($P \leq 0.05$ as significant). DEX independently increased ($P < 0.001$) InP with no effect of diet. With no interaction, Met sources or deficient diet did not influence gene expression of Claudin 1. DEX decreased ($P < 0.01$) Claudin 1 and 2. All three Met sources tended ($P = 0.069$) to upregulate expression of Claudin 2 compared with birds fed a deficient diet. With an interaction ($P < 0.05$), DL-Met and L-Met fed birds had a higher level of Claudin 3 only in DEX

injected birds. The expression of NF κ B remained unaffected. Diet and DEX interacted ($P < 0.001$) for expression of GLP-2 where unchallenged and challenged birds fed Met deficient diet had the highest and lowest expression, respectively. In conclusion, supplementation of Met in form of DL-Met, L-Met, and MHA-FA had minimal impact on InP and gene expression of tight junction proteins. While the effect of the tested leaky gut model was largely independent of the dietary treatments, no distinctive advantage of any of the sources of Met over another was found in relation to the tested gut barrier function parameters.

Key Words: inflammation; gut integrity; Methionine; dexamethasone; tight junctions

267 Efficacy of DL-Methionine and OH-Methionine utilization in broilers: a meta-analysis.

Elias Uddin^{3,2}, Henk van Lingen^{3,4}, Yves Mercier^{1*}, Paula da Silva-Pires^{3,5}, Dolores Batanon-Alavo¹, Friedrich Rouffineau¹, Ermias Kebreab³, ¹Adisseo France S.A.S., Malicorne, France, ²South Dakota State University, Brookings, United States, ³University of California, Davis, Davis, United States, ⁴Wageningen University & Research, Wageningen, Netherlands, ⁵Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

This work aimed to quantitatively assess the efficacy of DL-Methionine and OH-Methionine of broilers fed at or below requirements of Methionine. A literature search following the PRISMA procedure was applied in scientific databases without date restriction. This search resulted in a final database including 480 records of treatment means from 39 dose-response studies conducted from 1985 to 2019. The database contained information about the strain, experimental design, ingredient composition, diet nutritional values and growth performance. The nutrient composition of all diets was recalculated using the Practical Program for Formulation of Rations (PPFR, Garcia-Neto, 2008). Based on the age of the birds, the broiler database was divided into three subsets: starter, grower, and finisher phases. The database was analyzed using a Bayesian approach. This modeling approach was selected for several reasons among which 1) the pre-existing knowledge could be incorporated as prior information in the Bayesian model, which helps in estimating the requirement by restricting it to the range of the independent variable and 2) it facilitates fitting of complex models with large number of random variances. All model simulations were run in R statistical software. Firstly, the average daily gain (ADG) of the 3 broiler subsets was predicted from digestible Met or digestible sulfur amino acid using a 3-stage hierarchical model. The estimated digestible Methionine requirement were consistently greater for the quadratic-plateau compared to the linear-plateau model, which were 0.19 ± 0.01 vs. 0.13 ± 0.01 g/d for the starter, 0.50 ± 0.04 vs. 0.34 ± 0.02 g/d for the grower and 0.60 ± 0.04 vs. 0.51 ± 0.03 g/d for and finisher broilers, respectively. Secondly, based on

the estimated requirement, the 3 subsets were further divided to retain only observations below or equal to the breakpoint. Finally, using the new subsets within each phase, the ADG was fitted as response to the supplemental Met intake (= Met intake – Met intake of the basal, non-supplemented diet). No significant statistical difference was detected in the weight gain in response to the DL-Met and OH-Met intake, at or below the requirement. Consequently, favoring one form of Met over another may only depend on the availability, cost, ease of inclusion in the diet, and choice of the producer, but not on the growth performance.

Key Words: broilers; Meta-analysis; hydroxy-methionine; DL-Methionine; bio-efficacy

268 The digestible Sulfur amino acid requirement of 0 to 28 day-old turkeys is not affected by methionine source. Rob Shirley^{2*}, Dolores Batonon-Alavo², Yves Mercier¹, Jeffre Firman³, ¹*Adisseo France S.A.S., Malicorne, France*, ²*Adisseo USA, Inc., Woodstock, United States*, ³*Missouri Contract Poultry Research, Boonville, United States*.

This trial aimed (i) to determine the digestible sulfur amino acid (dSAA) requirements in the starter phase of Hybrid Tom turkeys fed increasing doses of DL-Methionine (DL-Met) or OH-Methionine (OH-Met) and (ii) to compare the effects of the two Met sources on the performance of turkeys. A total of 3,200 male Hybrid Tom turkeys were raised from 0 to 28 days in floor pens. They were placed at 20 birds/pen and 10 replicate pens/treatment. Treatments were based on a corn-soybean meal diet, formulated to be low in dSAA (Basal diet; 0.700% dSAA, 0.336% dMet). To the basal diet, DL-Met or OH-Met were added in 7 equimolar increments of dSAA (0.08% increments to 1.26% dSAA in the summit diet). Additionally, a diet sufficient in dSAA (1.03%) was fed as a Positive Control to ascertain that maximal growth occurred in the dSAA sufficient treatments (16 treatments in total). The respective diets were fed throughout the entire 28-day period. A one-way ANOVA was applied on the treatments for mean comparisons, and an ANCOVA model was applied to assess the effects of Met source and dSAA level. In addition, body weight gain (BWG) and feed conversion ratio (FCR) were analyzed using the 2-slope regression, broken-line and quadratic plateau models to determine the dSAA requirement for each Met source over the 28-d period. Although no significant differences in performance were observed between treatments for either DL-Met or OH-Met, all performance criteria were significantly affected by the level of dietary dSAA. Furthermore, no significant interactions were observed between Met source and dSAA level for all variables, demonstrating that DL-Met and OH-Met equally sustain turkey growth performance. Using the 2-slopes model for the BWG and FCR, the respective dSAA requirements were estimated at 1.07 and 1.01% for OH-Met and 1.07 and 1.00% for DL-Met. Analysis using the broken-line model for BWG gave slightly different values of 1.00% and 1.03% for OH-Met and DL-Met, respectively. For FCR,

the dSAA requirement was 0.99%, regardless of Met source. Using the Quadratic plateau model, the respective dSAA requirements for BWG and FCR using OH-Met were 0.89 and 0.93%, respectively, and 0.89 and 0.85% for DL-Met. Comparing the models above, the estimated 2-slope and broken-line model requirements are slightly above or match that of the Positive Control, which supplied 1.02% dSAA; the Quadratic plateau model resulted in a lower requirement, which typically overestimates requirements. In this study, the dSAA requirement estimates are similar between the two Met sources, independent of the equation used.

Key Words: requirement; DL-methionine; Turkeys ; Hydroxy methionine

269 Response of Ross 308 breeder hens to different levels of protein and available lysine. Léa Verrière^{1*}, Carole Margetyal¹, Olivier Gestin², Marie- Laure Godde², Claire Launay¹, ¹*ADM Animal Nutrition Neovia, Château-Thierry, France*, ²*ADM Animal Nutrition SERMIX, Saint-Nolff, France*.

Broiler breeder amino acids requirements during the very last part of the production cycle are globally not frequently investigated. Potential nutritional adjustments are therefore difficult to apprehend. A study was thus conducted to evaluate the effect of variable digestible lysine (digLys) and crude protein (CP) levels. A total of 138 Ross 308 broiler breeder hens were placed in individual cages and distributed in a completely randomized design among 6 treatments, with 23 replicates each. Treatments were provided from 58 to 65 weeks of age after a 4 weeks adaptation period. The experimental diets included 2 levels of total protein, with 3 corresponding levels of digLys : 0.36, 0.43, 0.50 and 0.43, 0.50, 0.61, for respectively, 11% and 13% CP. The other essential amino acids, methionine, threonine and tryptophane followed the CP level. Feed distribution was restricted to 139-140 g/d/hen. The feed consumption of each experimental unit was measured on a weekly basis. Corresponding laying performance (laying rate, egg weight, egg mass produced, feed conversion ratio per g of egg mass and per eggs, abnormal eggs) were assessed. Breeder hens were weighed at the beginning and at the end of the trial to assess the potential impact of treatments on body weights. Data were subject to a one-way ANOVA using a repeated measures model (gls). Means with significant differences were separated by Tukey's HSD test at $P < 0.05$. At both CP levels, laying rate was significantly improved with the second step of lysine, corresponding to an equivalent digLys : CP ratio (45 mg/g), with no improvement further. The highest laying rate thus achieved is significantly higher with 13% CP, compared to 11% CP (2.7 point ; $P < 0.001$). For a given digLys level, eggs tended to be heavier with 13% CP, but it is interestingly shown that 0.50% digLys level on 11% CP can enable to reach the same egg weight than 13% CP with 0.43% digLys. Egg mass produced and feed efficiency were gradually improved as the increases in CP

and lysine contents alternate. Unmarketable Dirty eggs are the lowest for the intermediate digLys:CP ratio (45 mg/g) reflecting a better balanced diet. From 58 to 66 weeks of age, weights of the birds on the 11% CP diet have decreased (-24g ; $P < 0.001$), whereas birds gained weight after receiving the 13% CP (between 39g and 73g, depending on the digLys). All together, these results show how protein and lysine are co-limiting factors: a good balance (namely around an digLys:CP of 45 mg/g) between these 2 nutrients help to optimize Ross 308 breeder hens performance.

Key Words: lysine; protein; breeders; Ross 308; lysine:protein ratio

270 Precise nutrition: Correlations between the KOH protein solubility test and the coefficients of *in vivo* digestibility of 15 amino acids beyond lysine and arginine in commercial lots of soybean meal that have undergone the Maillard reaction. Nelson Ruiz^{1*}, Carl Parsons², Benjamin Parsons², ¹Nelson Ruiz Nutrition, LLC, Suwanee, Georgia, United States, ²University of Illinois, Urbana, Illinois, United States.

At the 2022 International Poultry Scientific Forum (IPSF, Abstract M106), regression equations to estimate digestible lysine and digestible arginine in commercial soybean meal (SBM) using the KOH protein solubility (KOHPS) test were presented. The objective of this presentation is to further statistically analyze the correlations between KOHPS and the remaining 15 amino acids (AA). A total of 80 SBM samples were analyzed for KOHPS at Dairyland Labs, Arcadia, Wisconsin utilizing the newly standardized KOHPS method. Many of the samples had been overcooked and had undergone the Maillard reaction to varying degrees. These 80 samples came from a set of approximately 100 SBM samples previously analyzed for *in vivo* poultry amino acid digestibility and kept frozen during storage. The *in vivo* AA digestibility was determined utilizing the precision-fed rooster bioassay. A minimum of 4 cecectomized White Leghorn roosters per SBM sample were fasted for 24 h prior to being precision fed (crop intubation) 30 g. Excreta were collected for 48 h post feeding. The KOHPS values ranged from 86% to 13%. Linear and quadratic regressions of digestible AA coefficients on KOHPS were conducted using PROC GLM and broken line regressions were conducted using PROC NLIN in SAS. Regressions of digestible coefficients on KOHPS values were run for aspartic acid, threonine, serine, glutamic acid, proline, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, and tryptophan. Effects were considered significant at $P < 0.05$. Only total concentrations of lysine and arginine were reduced as a consequence of Maillard reactions. Although for all AA their digestibility decreased as KOHPS descended from 86% to 13%, only 2 AA plateaued at KOHPS values of 80% and above: digestible lysine and digestible arginine. Aspartic acid was the next AA with a KOHPS value at plateau of 70%. In other words,

the break point for the 1-slope broken line regression for digestible lysine occurred at 80% KOHPS (with a digestible lysine coefficient of 0.88), indicating that the minimum KOHPS value for SBM quality adequacy is 80% (IPSF, Abstract M106); therefore, none of the remaining AA are of significant concern from the quality of SBM stand point because their KOHPS values at plateau are well below 80% (ranging from 28 to 51%). In conclusion, only digestible lysine and digestible arginine are of concern for practical formulation purposes when KOHPS values are below 80%.

Key Words: soybean meal; KOH protein solubility; *in vivo* amino acid digestibility; 1-slope broken line regression; Maillard reaction

271 Dietary supplemental with methionine improves the production performance of breeding pigeons and the growth of squabs (*Columba livia*). Chen Zhong^{1*}, Di-qing Tong¹, Xiu-qi Wang¹, Chun-qi Gao¹, ¹South China Agricultural University, Guangzhou, China.

In the early stage of growth, squabs depend entirely on the nutritional supply from crop milk of breeding pigeons, but the mortality rate of squab is as high as 15% due to the lack of crop milk, which seriously restricts the rapid development of meat pigeon industry. Previous studies have shown that dietary methionine (Met) levels directly affect protein expression of methionyl-tRNA synthetase (MetRS) and protein synthesis of crop milk, but the exact mechanism is not precise. This study aimed to explore the mechanism of MetRS and JAK2/STAT5 signaling pathway mediating Met to regulate crop milk protein synthesis and provide theoretical basis and nutritional strategies for improving crop milk secretion. 216 pairs of white-feathered pigeons with similar reproductive performance and laying eggs on the same day and 864 squabs were randomly divided into 3 treatment groups. The control group was fed a basal diet (Met=0.55%) for the whole period (1 to 45 days), the Met-deficient group was fed a Met deficient diet (Met=25%) for the entire period, Met-supplement group was provided with Met deficient diet in the early period (1 to 13 days), and basal diet in the later period (14 to 45 days). During the experiment, the production performance of breeding pigeons and the growth performance of squabs were measured, and the crop milk and crop tissue samples were collected to measure the weight and thickness of the crop. Western blotting and immunofluorescence were used to detect the expression of marker proteins of proliferation and differentiation of crop epithelial cells, MetRS, and critical proteins of the JAK2/STAT5 signaling pathway. Duncan's multiple range tests were used to determine the differences among three groups using SPSS. The results showed that compared with the control group, Met deficiency significantly increased body weight loss, mortality, the prolonged interval between egg production, and decreased relative weight and thickness of crop, β -casein content in crop milk and average daily gain of squabs ($P < 0.05$). Dietary Met supplementation significantly improved the

performance of breeding pigeons and the growth performance of squabs, and promoted crop development and milk protein synthesis ($P < 0.05$). The expression levels and contents of PCNA, KRT19, MetRS, p-JAK2, p-STAT5, β -casein, α_{S1} -casein and WAP in crop were significantly increased, and the expression of JAK2 negative regulatory factor SOCS3 was significantly down-regulated ($P < 0.05$). In conclusion, Met supply can dramatically up-regulate the

expression of MetRS, crucial proteins in JAK2/STAT5 signaling pathways and promote the proliferation and differentiation of crop epithelial cells as well as the protein synthesis of crop milk.

Key Words: methionine; crop milk; Domestic pigeons; MetRS; JAK2/STAT5 pathway

Immunology, Health and Disease II

272 Gut microbiota-derived indole-3-carboxylate influences mucosal integrity and immunity through the activation of the aryl hydrocarbon receptors and nutrient transporters in broiler chickens challenged with *Eimeria maxima*. Inkyung Park^{1*}, Hyoyoun Nam¹, Doyun Goo¹, Samiru Wickramasuriya¹, Noah Zimmerman², Alexandra Smith², Thomas Rehberger², Hyun Lillehoj¹, ¹USDA-ARS, Beltsville, MD, 20705, Beltsville, Maryland, United States, ²Arm & Hammer Animal and Food Production, Waukesha, Wisconsin, United States.

Two studies were conducted to evaluate the effects of dietary indole-3-carboxylate (ICOOH) as a postbiotic on maintaining intestinal homeostasis against avian coccidiosis. In the first study, an *in vitro* culture system was used to investigate the effects of ICOOH on proinflammatory cytokine response of chicken macrophage cells (CMC), gut integrity of chicken intestinal epithelial cells (IEC), differentiation of quail muscle cells (QMC) and primary chicken embryonic muscle cells (PMC), and anti-parasitic effect against *Eimeria maxima*. Cells grown in the 24-well plates were treated with ICOOH at concentrations of 0.1, 1.0, and 10.0 µg. CMC was first stimulated by lipopolysaccharides (LPS) to stimulate innate immune response, and QMC and PMC were treated with 0.5 and 2% fetal bovine serum, respectively, before they were treated with ICOOH. After 18 h of incubation, cells were evaluated by RT-PCR for proinflammatory response of CMC, gut integrity of IEC, and muscle growth of QMC and PMC. In the second study, *in vivo* trials were carried out to evaluate the dietary ICOOH on disease parameters in *E. maxima*-infected broiler chickens. Four treatment groups, basal diet without infection (CON), basal diet with *E. maxima* (NC), ICOOH (10.0 mg/kg) with *E. maxima* (HI), and (4) ICOOH (1.0 mg/kg) with *E. maxima* (LO) were evaluated based on body weight changes on days 0, 7, 14, 20, and 22. All groups except the CON were orally infected with *E. maxima* on day 14. Jejunal samples were collected for lesion score, and the transcriptomic analysis of cytokines and TJ proteins. Data were analyzed using PROC MIXED in SAS. *In vitro*, ICOOH increased the expression of TJ proteins in IEC, and decreased IL-1β and IL-8 transcripts in the LPS-stimulated CMC. *In vivo*, chickens on HI diet showed reduced IL-1β, IFN-γ, and IL-10 expression, and increased expression of genes activated by aryl hydrocarbon receptors and nutrient transporters in *E. maxima*-infected chickens. In conclusion, these results demonstrate the beneficial effects of dietary ICOOH on host intestinal immune responses and gut barrier integrity in *E. maxima*-infected chickens. Furthermore, the present finding supports a notion to use microbial metabolites as novel feed additives to reduce antibiotic resilience in animal agriculture.

Key Words: antibiotic alternatives; gut health; *Eimeria maxima*; indole-3-carboxylate; intestinal metabolites

273 Evaluation of a phytogetic compound, Orego-Stim®, in broiler chickens during an *Eimeria maxima* challenge. Matthew Jones^{2*}, Wendy Wakeman³, Elizabeth Krushinskie³, Laura Corbett³, Allberta Fuller¹, Charles Hofacre², ¹University of Georgia, Athens, Georgia, United States, ²Southern Poultry Research Group, Inc., Watkinsville, Georgia, United States, ³Anpario plc, Workson, United Kingdom.

Coccidiosis impacts both broiler welfare and performance. In addition to creating pathology on an individual basis, *Eimeria* species, like *E. maxima*, are also pathogens that predispose broilers to necrotic enteritis in the presence of a toxigenic strain of *C. perfringens*. Some phytogetic products influence *Eimeria* species and may be beneficial in mitigating conditions involving this protozoal parasite. In a battery cage experiment, one phytogetic product, Orego-Stim® (OS), was tested at multiple levels (600 g/MT, 800 g/MT, and 1000 g/MT) relative to positive and negative control groups. Each of the five treatments was represented by nine replicate cages of 10 male Ross 708 chicks. The wire floor in the battery cages allowed excreta to pass into a tray below each cage floor. Challenged groups were orally gavaged with *E. maxima* on day of test (DOT) 8 at a concentration of 75,000 sporulated oocysts/mL. Paper was kept in each cage to DOT 14 to encourage *Eimeria* cycling. On day 14, gross lesions were evaluated as described by Johnson and Reid 1970 from 3 birds per cage, and excreta was collected from each cage for oocyst/gram (OPG) enumeration. Excreta was also collected on DOT 21, 25, and 28. The birds and feed were weighed on day 0, 14, 21, and 28 to evaluate growth performance. Data were subjected to an ANOVA and means were compared using an LSD test at a *P*-value of 0.05. On DOT 14, the *E. maxima* challenge separated the body weight gain, feed conversion, and feed intake between the challenged and unchallenged controls. All OS challenged groups had greater body weight gain than the challenged group on day 14 at the peak of this severe challenge. Day 14 weight gain for OS at 600g/MT, 800g/MT and 1000g/MT were 0.290 kg, 0.278 kg, and 0.276 kg, respectively, while the challenged control group weight was 0.252 kg. Adjusted feed conversion was statistically similar between the challenge OS groups and the negative control. At day 21, OS at 600 g/MT had greater body weight and feed consumption than the challenged control. At 28 days the OS at 600 g/MT maintained greater feed consumption than the challenged control. Gross lesions and total OPG counts were not significantly different between challenged groups. The presence of oocysts at DOT 28 in challenged groups suggests that the birds had a lingering *Eimeria* challenge. The OPG shedding pattern may indicate that OS influenced later *E. maxima* cycling. The body weight gain improvement at the peak *E. maxima* challenge indicates that OS mitigated the severe effects from the coccidia cycling.

The sustained feed intake also suggests that Orego-Stim helps broilers recover from the intestinal challenge, especially at the 600 g/MT dose, and continue to eat.

Key Words: Eimeria; Coccidia ; Phytogetic; Enteric Health

274 Investigation of chicken tumor necrosis factor- α (TNF- α) and colony stimulating factor (CSF) using new sets of monoclonal antibodies specific for poultry TNF- α and CSF in broiler chickens infected with Coccidiosis. Hyun Lillehoj^{1*}, ¹USDA, Beltsville, Maryland, United States.

Tumor necrosis factor- α (TNF- α) is a type II transmembrane protein with either membrane-bound or soluble forms and is a prototypical member of the TNF superfamily. TNF- α is a pleiotropic cytokine associated with the regulation of systemic inflammation and host defense. Macrophage colony-stimulating factor-1 (M-CSF-1 or CSF-1) is a hematopoietic growth factor that stimulates the survival, proliferation, and differentiation of the mononuclear phagocyte lineage and is involved in bone metabolism, fertility, pregnancy, inflammatory processes, and homeostasis. These cytokines are important mediators of host innate immune response against pathogens and there is lack of information on these cytokines in poultry diseases such as coccidiosis and necrotic enteritis (NE). Here, we report the functional characterization and immunomodulatory properties of chTNF- α and chCFF-1 using a panel of newly developed mouse monoclonal antibodies (mAbs). Furthermore, we used these antigen capture assays to detect their levels in coccidiosis and necrotic enteritis (NE). Detection of these important chemokines in coccidiosis and NE will facilitate our understanding of host-pathogen immunobiology and facilitate the development of novel therapeutic strategies. Collectively, our results will improve our understanding of the functional characteristics of chTNF- α and chCSF-1, and these novel mAbs will serve as valuable immune reagents to inform on inflammatory responses and disease pathogenesis in the fundamental and applied studies of avian species.

Key Words: Coccidiosis; Sandwich ELISA; Innate immunity; Tumor necrosis factor; Colony stimulating factor

275 Interaction of microbiota and mucosal immunity in broilers infected with *Eimeria tenella*. Janghan Choi^{1*}, Woo Kim¹, ¹University of Georgia, Athens, Georgia, United States.

Eimeria tenella causes hemorrhagic coccidiosis in ceca, the main area for the microbial fermentation in broilers. Infection of *E. tenella* may alter cecal microbiota potentially via negatively affecting mucosal immunity and increasing the digesta protein concentrations by shedding host cell debris. The purpose of the study was to investigate the effects of *E. tenella* infection on microbiota, mucosal immunity, and digesta protein concentration in the ceca of

broilers. A total of 360 fourteen-day-old male Cobb 500 broilers were allocated to 5 treatments with 6 replicates of 12 birds per cage. The 5 treatments were oral administrations of sporulated *E. tenella* as following dosages: T0: 0, T1: 6,250, T2: 12,500, T3: 25,000, and T4: 50,000 oocysts. Orthogonal polynomial contrasts were used to evaluate the significance of linear or quadratic effects of different *E. tenella* inoculation dosages. *E. tenella* infection tended to reduce alpha diversity indices including Shannon_entropy ($P = 0.07$) and Pielou_evenness ($P = 0.05$) in the cecal microbial communities. *E. tenella* infection significantly increased the relative abundance of phylum Proteobacteria ($P < 0.05$) and significantly decreased the relative abundance of phylum Firmicutes ($P < 0.05$). The relative abundance of family Enterobacteriaceae was linearly increased due to *E. tenella* infection ($P < 0.05$). While the protein concentration of the cecal content was linearly increased ($P < 0.05$), the concentration of secretory immunoglobulin A (sIgA) in the cecal content was linearly decreased due to *E. tenella* infection ($P < 0.05$). The number of goblet cell per crypt and goblet cell density were linearly reduced in the ceca due to *E. tenella* infection ($P < 0.05$). *E. tenella* infection tended to linearly decrease relative mRNA expression of antimicrobial peptides including avian β -defensins 9 (AvBD9; $P = 0.1$) and liver-expressed antimicrobial peptide 2 (LEAP2; $P = 0.08$) in the cecal tissues. Therefore, *E. tenella* infection negatively altered cecal microbiota and increased the relative abundance of the specific phylum and family that contain a wide variety of pathogenic bacteria via impairing cecal mucosal immunity and increasing protein concentrations in the cecal content in broilers.

Key Words: broilers; microbiota; Eimeria tenella; mucosal immunity

276 Searching for a cure for Histomoniasis: A meta-analysis of oral supplementations on reducing the morbidity and mortality of turkeys directly infected with *H. meleagridis*. Christina Sigmon^{1*}, Jinqian Wang², Harshavardhan Thippareddi², Robert Beckstead³, Chongxiao Chen¹, ¹North Carolina State University, Raleigh, North Carolina, United States, ²University of Georgia, Athens, Georgia, United States, ³Ceva Animal Health, Lenexa, Kansas, United States.

Histomoniasis, caused by *Histomonas meleagridis*, results in high mortality in turkeys and significantly impacts turkey production. Currently, there is no treatment available. A research model was developed and used in our lab to screen potential oral supplements on the effectiveness of reducing the morbidity and mortality of turkeys directly infected with *H. meleagridis*. In this model, turkey poults were infected with 100,000 histomonas cells/bird at ~ 2 weeks, and the trial was terminated at ~ 4 weeks. Infection rate and mortality were calculated. Cecal and liver lesions caused by *H. meleagridis* infection were scored on a 0-4 scale, with

4 being the most severe. Since 2016, we have screened products from 28 companies, tested 132 products in 5 categories (Functional carbohydrates, Plant extracts, Prototype, Minerals, Others: fatty acids, coccidiostats, prebiotics/probiotics/postbiotics, and Mixture: mix two or more products falling in the above 4 categories). 28 products were tested at different levels. A total of 196 trials were conducted in our lab. All data were analyzed using R version 4.0.2 (R Core Team, 2021). The infection and mortality rates were transformed using logit transformation. Metafor package in R was used to conduct the meta-regression on infection and mortality rate. Ceca and liver scores were regressed using lm function in R. In comparison to control groups, the meta-regression results suggested that oral supplementation of Functional carbohydrates and Plant extracts reduced the log odds ratio of mortality by 1.40 ($P<0.05$) and 0.55 ($P=0.10$). However, Others and Prototype slightly increased the log odds ratio of infection rate by 0.89 and 0.56 ($P<0.05$), respectively. For ceca score, Functional carbohydrates, Plant extracts, and Others could reduce the ceca score of *H. meleagridis* infection by 0.93, 0.45, and 0.39, respectively ($P<0.05$). While prototype only showed a trend to reduce the ceca score by 0.21 ($P<0.1$). Supplementation of Plant extracts or Others could reduce the liver score by 0.34 and 0.42 ($P<0.05$), respectively. Meanwhile, Functional carbohydrates showed a trend to reduce the liver score by 0.48 ($P<0.1$). In summary, the products in Minerals, Functional carbohydrates, and Plant extracts category that were tested in our lab showed a higher potential in reducing the morbidity and mortality of turkeys directly infected with *H. meleagridis*. Even though some products showed different levels of reduction in morbidity and mortality, a therapeutic product is needed to resolve histomoniasis.

Key Words: meta-analysis; turkeys; *Histomonas meleagridis*; oral supplements; morbidity

277 A formulation of essential oils, *Yucca schidigera* pulp and polyphenols alters *Eimeria* pathogenicity *in vitro* and reduces coccidiosis impact in both *Eimeria*-vaccinated or non-vaccinated broilers. Jonathan Pierron², Bertrand Medina³, Ivan Girard³, Carl Julien^{1*}, ²CRSAD, Deschambault, Quebec, Canada, ²Université Laval, Québec, Quebec, Canada, ³Probiotech International Inc., St-Hyacinthe, Quebec, Canada.

The aim of this study was to evaluate the effect of a formulation of essential oils, *Yucca schidigera* pulp and polyphenols (Synbiotec® (Syn), Probiotech International Inc.) as an anticoccidial alternative and as part of a bio-shuttle program in *Eimeria*-challenged birds. *In vitro* tests (oocyst sporulation, sporozoite viability and host cell invasion, 3 replicates) and two trials in combination or not with *Eimeria* vaccination on coccidiosis-challenged broilers (55 birds/pen, 9 replicates) were conducted. Trial#1 involved 1,980 Ross 308 males assigned to: 1) non-infected (NI); 2) *Eimeria*-infected (EI); 3) EI+decoquinate/zoalene

shuttle program (DZ); 4) EI+500 ppm Syn for 0-20d; 250 ppm for 20-34d (SYN500). In trial#2, 1,485 *Eimeria*-vaccinated (IMMUcox3®, CEVA) birds were assigned to: 1) NI; 2) EI; 3) EI+250 ppm Syn for 0-10d; 500 ppm for 10-20d; 250 ppm for 20-34d (SYN250). *Eimeria* infection was induced by an oral administration at d14 of 2×10^5 sporulated oocysts of mixed *Eimeria* species. Data were analyzed by a mixed model including treatment and barn section as fixed and random effects, respectively, and by Kruskal-Wallis tests. *In vitro* tests showed a dose-response effect of Syn (0.5, 12.5, 50 and 100 µg/mL) on partially decreasing sporozoite viability and host cell invasion, reaching -32% and -54%, respectively ($P<0.0001$), whereas zoalene, monensin and amprolium exposures reduced viability and invasion by 60% et 90% respectively at the same dosages ($P<0.0001$). Syn and anticoccidial exposures reduced oocyst sporulation by 80% ($P<0.0001$). In trial#1, EI reduced growth performances and increased oocyst excretion, intestinal lesions and mortality rates overall, compared to NI, whereas DZ palliated these infection impacts. SYN500 increased body weight (BW) at d34 (2.39 vs 2.26 kg, $P=0.0007$), and improved the overall feed conversion ratio (FCR) (1.38 vs 1.45, $P=0.0080$), without affecting mortality rates overall (9.5 vs 14.1%, $P=0.2706$) nor oocyst excretion or intestinal lesions, compared to EI. In trial#2, EI also reduced growth performances, oocyst excretion and intestinal lesions, compared to NI. SYN250 increased BW at d10 (250 vs 242 g, $P=0.0379$) and at d20 (808 vs 734 g, $P=0.0030$), and improved FCR during 0-10d (1.17 vs 1.23, $P=0.0001$), 10-20d (1.32 vs 1.50, $P=0.0021$) and overall (1.30 vs 1.33, $P=0.0348$), compared to EI. SYN250 reduced 7 dpi intestinal *E. tenella* lesion score (0.72 vs 1.33, $P=0.0484$), but not the total oocyst excretion, compared to EI birds. Overall, this tested botanical formulation can be used as an anticoccidial alternative or can be introduced in control strategies based on vaccination without anticoccidials to secure broiler performances.

Key Words: Broilers; Intestinal health; Conventional production; Bio-shuttle program; Botanical extracts

278 Evaluation of performance, lesion scores, intestinal integrity, and gut microbiome in broiler chickens challenged with *Eimeria* spp.: a model to evaluate alternatives to drug-based anticoccidials. Danielle Graham^{1*}, Makenly Coles¹, Jianmin Chai², Jiangchao Zhao¹, Aaron Forga¹, Juan Latorre Cardenas¹, Maria Trujillo Peralta¹, Roberto Cuesta¹, Victor Petrone³, Guillermo Tellez-Isaia¹, Billy Hargis¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Foshan University, Foshan, China, ³Universidad Nacional Autonoma de Mexico, Cuautitlan Izcalli, Estado de Mexico, Mexico.

Anticoccidial drugs and live vaccination have been used to prevent coccidiosis in commercial broiler flocks. The purpose of the present study was to establish a coccidiosis challenge model with *E. acervulina* (EA), *E. maxima* (EM)

and *E. tenella* (ET) to evaluate the effect of natural anticoccidial alternatives on performance, lesion scores, intestinal integrity, and gut microbiome composition in broiler chickens under laboratory settings. Treatment groups included: 1) non-challenged control (NC, n=5) and 2) challenged control (PC, n=7) with 20 animals/pen. Pen weights were recorded at d0, d16, d31, d42, and d52. Feed intake was measured at d16, d31, d42, and d52 to calculate feed conversion ratio (FCR). At d18, chickens were orally challenged with 200 EA, 3,000 EM, and 500 ET sporulated oocysts/chicken. At d24 (6d post-challenge), intestinal lesion scores were recorded for 4 chickens/pen (n=20 for NC; n=28 for PC). Additionally, fluorescein isothiocyanate dextran (FITC-d) was orally administered to evaluate impact of mixed *Eimeria* spp. challenge on intestinal permeability. Ileal and cecal contents were also collected for 16S rRNA sequencing and microbiome analysis. Significant ($P<0.05$) differences in average BWG and FCR between NC and PC group were observed from d16-31 with the NC having markedly higher BWG and lower FCR compared to the PC. The PC had significantly higher lesion scores for EA, EM, and ET compared to the NC. Increased intestinal permeability in the PC, as measured by serum FITC-d levels 6d post-challenge was attributed to severity of intestinal lesions. The cecal microbiome of the PC was significantly affected by *Eimeria* spp. challenge with decreased alpha diversity, including Shannon Index and the number of Observed Amplicon Sequence Variants (ASVs), compared to NC. The PC showed a distinct cluster based on Bray-Curtis and Jaccard distances. Linear discriminant analysis Effect Size (LEfSe) was employed to identify bacterial biomarkers for each group. *Clostridium sordelli* (ASV10), *Clostridium butyricum* (ASV11), *Ruminococcaceae* (ASV51), and *Bacteroidaceae* (ASV18) were enriched in the ileal content of the PC compared to the NC. Additionally, the abundance of *Clostridium perfringens* (ASV1, ASV12), *Enterobacteriaceae* (ASV8), *Clostridiales* (ASV23), and *Ruminococcaceae* (ASV81, ASV444, ASV92) was higher in the cecal content of the PC. These data indicate that *Eimeria* spp. challenge negatively impacts intestinal integrity leading to decreased performance during the challenge period and alters the ileal and cecal microbiome composition 6d post-challenge. This model can be used to assess the efficacy of natural alternatives to control coccidiosis in broiler chickens.

Key Words: broiler; coccidiosis; microbiome; *Eimeria*; intestinal permeability

279 Bone marrow histiocytes of lame ducklings resemble hemophagocytic histiocytes (HLH). Paul Cotter^{1*}, B. Fetrow², ¹Cotter Laboratory, Arlington, Massachusetts, United States, ²Maple Leaf Farms, Leesburg, Indiana, United States.

Hemophagocytic lymphohistiocytosis (**HLH**) is a rare human disease resulting from uncontrolled inflammation. Alternative names are “hemophagocytic syndrome”,

“macrophage activation syndrome” and “cytokine storm”. Both familial and acquired forms are known and recently HLH has been recognized as a complication to COVID infection. In other instances, multiple viral infections (EBV and parvovirus) have been identified in HLH. The diagnosis of HLH requires fulfillment of 8 clinical criteria based on symptomology and laboratory findings. Here the **purpose** was to demonstrate HLH like cells in the bone marrow of lame ducklings. HLH will be established using cytological criteria; hemophagocytosis in bone marrow (**BM**). As a working **hypothesis** histiocytes containing only hemosiderin, effete RBC, or debris are homeostatic (**ho**); those with non-effete RBC, other leukocytes, or thrombocytes are pathologic (**pa**). Fracture of cytoplasm, elaboration of pseudopods, and cytoplasmic shedding will indicate a histiocyte as a **pa** type. Such cells are presumed to represent the duck equivalent of HLH cells of humans. **Method:** Lame ducklings were identified by experienced technical service personnel and femur bone marrow touch preparation slides were prepared on farm. After air-drying and post fixing in EtOH, slides were stained using an in-house version of Wright’s method followed by a brief secondary exposure to Giemsa. Representative cells were photographed at 100x magnification. The **results**; BM sampled from lame ducklings between 7 and 31 days displayed giant phagocytic histiocytes (**gh**) and giant granulated histiocytes (**ggh**) containing effete or intact RBC, polychromatic RBC, thrombocytes, and heterophils. Examples of representative microscopic fields containing **gh** and **ggh**, classified as either pathologic (**pa**) or homeostatic (**ho**), based on cytology are the subject. These cells bear a resemblance to “strawberry cells” of humans afflicted with (**HLH**). It is likely they are the consequence of a combination of polymicrobial and fungal (*Hemomycetes avium*) infections.

Key Words: hemophagocytic lymphohistiocytosis; histiocyte; duckling; polymicrobial infection; *Hemomycetes avium*

280 Chicken alloantigen D is CD99. Robert Taylor^{1*}, Wioleta Drobik-Czwaro³, Janet Fulton², ¹West Virginia University, Morgantown, West Virginia, United States, ²Hy-Line International, Dallas Center, Iowa, United States, ³Warsaw University of Life Sciences, Warsaw, Poland.

Alloantigens are expressed on the cell surface of some, but not all, members of a species. There are 13 known alloantigen systems found on chicken red blood cells. The responsible gene or genomic region is known for only 3 of these systems, *A* (C4BPM; complement 4 binding protein, membrane bound), *B* (MHC; Major Histocompatibility Complex) and *E* (FCAMR; Fc fragment of IgA and IgM receptor), the sole candidate that fit multiple data sets. Systems *A* and *E* are linked, 89 Kbp apart on chicken microchromosome 26, whereas the *B* system is on microchromosome 16. This study sought to identify the gene and chromosomal location for alloantigen *D*. Diverse

sources including lines with known *D* system alleles and genomic sequences were utilized to identify the *D* system candidate gene. These stocks encompassed inbred lines UCD001, UCD003, IAH-61, IAH-72, and IAH-RHC plus elite lines from Hy-Line International. In addition, DNA samples representing known *D* types from the Northern Illinois University (NIU) DNA bank and specific lines from Hy-Line International were used. A genome wide association analysis using pooled NIU DNA bank samples having known *D* system phenotypes and Affymetrix 600K chicken SNP chip genotypes revealed a strong peak on chicken chromosome 1 at 1.3-1.4 Mb (Galgali6). This same region was confirmed with 54K SNP genotypes obtained from an independent Hy-Line International DNA sample set having known *D* system alleles. Selective criteria were established to identify candidate genes including; cell surface expression, SNP contained in exons, and SNP causing possible change in the protein. The CD99 (Xg blood group) gene met these criteria as well as matching the SNP haplotypes for *D* serologically defined alleles. Independent samples were used to confirm that the fit of specific CD99 SNP haplotypes was predictive for specific *D* system alleles. The corresponding gene is found on the human X chromosome overlapping with the pseudoautosomal region. The gene is involved in transmembrane protein transport and leukocyte migration. The availability of DNA based testing allows for easier detection of *D* system variation and its' impacts on avian immunity.

Key Words: alloantigen; sequence; Xg blood system; membrane transport; snp

281 Exposure to heat stress alters the structural integrity of the enteric nervous system in the broiler chicken ileum. Valentina Caputi^{1*}, Komala Arsi², Kelsy Robinson³, Annie Donoghue², Joshua Lyte², ¹University of Arkansas, Fayetteville, Arkansas, United States, ²USDA-ARS Poultry Production and Product Safety Research Unit, Fayetteville, Arkansas, United States, ³USDA - ARS, Starkville, Mississippi, United States.

Objective: Heat stress (HS) in broiler chickens severely affects their gastrointestinal (GI) tract, increasing intestinal permeability and inflammation, and favoring the colonization of bacterial foodborne pathogens. The enteric nervous system (ENS) is the intrinsic innervation of the GI tract and plays a fundamental role in regulating secretion, absorption, and motility, as well as host immune surveillance against pathogenic bacteria. The effect of HS on broiler chicken ENS integrity is unknown, and this represents a novel research approach for reducing foodborne pathogens in poultry through the investigation of the neurobiological mechanisms of stress-induced perturbations of gut health. Therefore, the present study evaluates whether HS determines structural alterations in broiler chicken ENS. **Experimental design:** Male broiler chicks were randomly divided into control or HS groups, placed in pens, and kept under standard environmental

conditions. At 6 weeks of age, a 12-h daily cyclic HS (35°C) was applied for either 1 day or 6 consecutive days to the HS group, whereas control chickens were maintained under standard age-appropriate conditions. Birds were euthanized following either 1 day or 6 days of HS. Birds from the control group were sacrificed on the same days and age as those from the HS group. **Methods:** The distribution of HuC/D (pan-neuronal marker) and neuronal nitric oxide synthase (nNOS; nitrergic neurons marker) was assessed in ileum wholemount preparations (N=6 birds/group) by performing tissue clearing and double-labelling confocal immunofluorescence. The integrity of glial fibrillary acidic protein (GFAP; enteric glial marker) and ionized calcium-binding adaptor molecule 1 (Iba1; enteric muscular macrophages marker) was determined by confocal microscopy in ileal frozen sections. **Statement of statistical analyses:** Data were analyzed using two-tailed unpaired Student's t test with Welch's correction. **Results:** HS caused a marked distortion of the dendritic and axonal processes of nNOS-containing neurons with no changes in the number of HuC/D⁺ neuronal cells in the myenteric ganglia of broiler chickens exposed to HS for 1 or 6 days. In the neuromuscular compartment of 1-day HS chickens the density of GFAP⁺ processes was significantly increased (p<0.05), and these changes persisted after 6 days of HS. The immunoreactivity of Iba-1 was not affected by HS in the chicken ileum. **Conclusions:** HS caused structural alterations of enteric neurons and glial cells in the broiler chicken ileum. These findings warrant future studies aimed at identifying ENS-related biomarkers of stress-induced susceptibility to intestinal inflammation and foodborne pathogen colonization in poultry.

Key Words: stress; poultry; intestine; enteric nervous system; neurobiology

282 Antiviral activities of phytogetic substances against H5N1 highly pathogenic avian influenza virus *in vitro*. Xiaodan Zhou^{1*}, Tobias Aumiller¹, Karola Wendler¹, ¹Delacon Biotechnik GmbH, Engerwitzdorf, Oberösterreich, Austria.

Highly pathogenic avian influenza virus H5N1 is a global threat to poultry and human health. Although vaccines and anti-viral drugs are available, their cost and potential for resistance development have raised interest in alternative options. Antiviral and pharmacologically active compounds derived from plants are in interest in this regard. Therefore, in the present study we evaluated the antiviral activities of marshmallow extract (ME), dandelion extract (DE), lemongrass essential oil (LEO) and oseltamivir phosphate (OP) against H5N1 *in vitro*. Experiment (Exp) 1: MDCK cells were incubated with ME, DE, LEO or OP at their maximum non-cytotoxic concentrations for 12 h (1000, 1000, 500 and 23.44 µg/mL of ME, DE, LEO and OP, respectively) followed by addition of H5N1 at different multiplicity of infection (MOI) ratios for 1 h. Then the supernatant was replaced with

DMEM containing 2% FBS and incubated 2 days to determine cytopathic effects (CPE). The MOI of H5N1 where treatments could reduce CPE was considered as the maximum inhibition dose (MID). Exp 2: Exp 1 was repeated with H5N1 at MID. Median tissue culture infective dose (TCID₅₀) was determined. Exp 3: Exp. 2 was repeated with LEO at different concentrations (10, 100 and 500 µg/mL). All treatments were measured in triplicates and each experiment was run 3 times independently. Statistical analysis was performed using SAS 9.4 (SAS, SAS Institute, Inc., Cary, NC, USA). For all data, the GLM procedure with Tuckey HSD test was used. Statistical significance was declared at $P \leq 0.05$. Results: In Exp 1, ME, DE, LEO and OP decreased ($P < 0.05$) CPE with H5N1 at MOI 10⁻², 10⁻³, 1 and 1, respectively. In Exp 2, Cells treated with OP reduced ($P < 0.05$) TCID₅₀ at MOI 1, 10⁻², 10⁻³ by 18.7%, 26.5% and 26.2% compared to NC, respectively. LEO reduced ($P < 0.05$) TCID₅₀ by 64% and 56% at MOI 1 compared to NC and PC, respectively. ME and DE had no effect on TCID₅₀ at MOI 10⁻² and 10⁻³. In Exp 3, LEO at 10, 100 and 500 µg/mL decreased the TCID₅₀ at MOI 1 by 4.4% ($P = 0.44$), 55.7% ($P < 0.0001$) and 64.1% ($P < 0.0001$), respectively, compared to the NC. These results indicate that LEO impedes the structural changes of MDCK cells by reducing viral titers in a dose-response manner. In conclusion, LEO has the highest potential among tested phytogenic substance to inhibit the replication of H5N1 highly pathogenic avian influenza virus in a pre-incubation manner *in vitro*.

Key Words: avian influenza; H5N1; phytogenic substance; antiviral

283 Dietary Vitamin K supplementation modulates Lipopolysaccharide-induced growth performance, inflammatory response, immune dysregulation and intestinal injury at an early age in broiler. Yujiao Lai^{1*}, Jiaqi Lei¹, Kewei Yu¹, Zhuli He¹, Yitao Mao¹, Tanfang Li¹, yang He¹, Qihang Hou¹, Chaoyong Liao¹, Yanbo Ma², Yuming Guo¹, Bingkun Zhang¹, ¹China Agricultural University, Beijing, China, ²Henan University of Science and Technology, Beijing, China.

Vitamin K (VK) is kind of necessary micronutrients used in poultry feed. Little information is available about the effect of VK supplementation on growth performance, inflammatory response, immune dysregulation and intestinal injury, ileal transcriptional profile of chicks challenged by Lipopolysaccharide (LPS), let alone

comparison with regulatory role of VK3 and VK2. A total of 396 healthy 1-day-old arbor Acres broilers were randomly divided into 6 treatment groups, with 6 replicates of 11 birds each cage. Experimental treatments were designated as follows: chicks received a basal diet (CON), LPS-challenged chicks fed with a basal diet (LPS), LPS-challenged chicks fed with a basal diet supplemented with 1, 3, 9 mg/kg VK₂ levels (VK₂L, VK₂M, or VK₂H), respectively, LPS-challenged chicks fed with a basal diet supplemented with 3 mg/kg VK₃ levels (VK₃). The results were calculated by ANOVA with SPSS Statistics 26 (SPSS Inc., Chicago, IL). The results showed that intraperitoneal injection of LPS significantly decreased growth performance, induced hypertrophy of spleen and liver and atrophy of bursa of fabricius, liver damage, decrease of serum levels of immunoglobulins, ucOC and ucMGP, and intestinal inflammation and injury in broilers. VK₂ supplementation effectively alleviates growth performance impairment, inflammatory reactions and intestinal injury induced by LPS. Transcriptomic analysis identified 899 differently expressed genes (DEGs) (323 upregulated and 576 downregulated) in the LPS group compared with the CON group, 96 DEGs (58 upregulated and 38 downregulated) in the VK₂ group compared with the LPS group and 67 DEGs (28 upregulated and 39 downregulated) in the VK₃ group compared with the LPS group (P adjusted value < 0.05 , |fold change| > 1.5), respectively, which revealed that LPS challenge altered the gene expression and signaling pathways of Toll-like receptor and MAPK signaling pathway in the ileum of LPS-treated broilers, while VK₂ modulated MAPK signaling pathway and VK₃ may improve energy utilization by promoting the tricarboxylic acid cycle. In addition, dietary VK supplementation improved the effects of LPS on intestinal morphology, blood biochemical parameters, oxidative stress, and intestinal inflammation of broilers. Therefore, dietary VK supplementation can modulate the RNA expression profile and immune dysregulation, attenuate inflammatory response and intestinal injury in LPS-challenged broilers, thus improve the growth performance of broilers with intestinal injury. Conclusively, VK₂ and VK₃ exert similar role in LPS-induced inflammatory response, immune dysregulation and intestinal injury, and VK₂ showed better regulation effect than VK₃ in broilers.

Key Words: transcriptome; vitamin K; Lipopolysaccharide; inflammatory response; intestinal injury

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284 Effect of probiotic and antibiotic growth promoter in water on microbiota in broiler chickens.

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The gastrointestinal tract (GIT) microbiota plays an important role in nutrient absorption, immune system development, and disease resistance. The microbiota can be transiently modulated by antibiotic growth promoters (AGP), probiotics (P), prebiotics and other additives. The aim of this study was to determine the effects of P supplementation on microbiota in broilers in comparison to AGP treatment. Ross 708 hatchlings (550) were fed standard corn-soybean meal diet (C) and provided one of the following treatments in water: (1) BMD Soluble (Bacitracin methylene disalicylate, Alpharma, equivalent to 50g/ton of feed, AGP) or (2) FloraMaxB11 (Pacific Vet Group, 10⁶ cfu/ml water) for first three d (P3), 10 d (P10) and 35 d (P35) post-hatch (PH). Body weight (BW) and feed intake (FI) were recorded during sampling at 3, 10, 21 and 35 d PH. To evaluate the luminal (L) and mucosal (M) bacterial populations, samples of digesta and epithelial scrapings were collected, respectively, from the jejunum (JE), ileum (IL) and ceca (CE). BW, FI and feed conversion ratio (FCR) were calculated and analyzed using SAS. The microbiota was determined by sequencing of the V3-V4 region of bacterial 16S rRNA (Illumina). Alpha and beta diversity were analyzed using Kruskal-Wallis and PERMANOVA, respectively (QIIME 2). Differential abundance of bacterial taxa was analyzed using Linear Discriminant Analysis Effect Size (LEfSe). Metagenomic functions were predicted using PICRUST2. BW, FI and FCR were not affected by AGP or P treatment ($P>0.05$). However, numerically, P3 birds were on average 148 g and 42 g heavier than C and AGP birds, respectively. Several alpha diversity indexes were significantly ($P<0.05$) affected by treatments in both L and M bacterial populations of JE, IL, and CE. Beta diversity were affected ($P<0.05$) by treatment in all bacterial populations except for IL-M and JE-L. Taxonomic analysis revealed significant ($P<0.05$) changes in relative abundance of some bacterial genera and species, including *Escherichia coli* and *Clostridium celatum* (CE-L), *Lactobacillus*, *Oscillospira* and *C. celatum* (CE-M), *Clostridium*, *SMB53*, *Lactobacillus reuteri* and *C. celatum* (IL-M) and *Ralstonia* and *Lactobacillus* (JE-M). LEfSe analysis showed that relative abundance of genus *Weissella* was decreased in AGP birds in all parts of GIT in comparison to non-AGP birds (C, P3, P10 and P35). Predicted function of microbiota was mostly affected in IL-M, followed by IL-L, CE-M, CE-L and JE-M. In conclusion, probiotic treatment for the first three days post-hatch seems to have some effect on BW. Changes in microbiota function and composition were dependent on the GIT localization and to some extent on the duration of P treatment.

Key Words: AGP; chicken; microbiota; probiotics; GIT

285 Ecology of avian *Escherichia coli* in health and disease using a refined typing scheme.

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Avian pathogenic *Escherichia coli* (APEC) belong to a broader pathotype known as extraintestinal pathogenic *E. coli*, and APEC is the causative agent of colibacillosis affecting all production bird types. We recently assessed more than 3,800 genomes from commercial turkey and broiler production, including both clinical and cecal isolates, and utilized this knowledge in combination with virulence assays to develop a refined approach to typing APEC in poultry production. Using this revised approach, we examined the ecology of avian *E. coli* across multiple commercial turkey flocks within a vertically integrated system. We hypothesized that differences exist in *E. coli* ecology and dominant strains across different locations in the bird, and that extensive diversity exists across a single farm system. To address these questions, we performed sampling and characterization of the litter environment and trachea/cloaca of individual birds from 500 samples with up to 5 colonies per sample, totaling more than 2,500 isolates. These isolates were all characterized using a revised APEC typing scheme, and 150 isolates were further characterized using whole genome sequencing. We found that avian *E. coli* is extremely diverse in these locations, yet a small subset of clones is responsible for the vast majority of clinical disease. Extensive variability in strains occurred from farm-to-farm and over time. Dominant APEC clones were more likely to be identified in the respiratory tract of healthy birds, compared to the gastrointestinal and litter environments. Still, the identification of high-risk clones that dominate the clinical landscape was rare in the gut and respiratory environments of healthy birds. Overall, this study increases our knowledge of the overall ecology of *E. coli* in poultry production, and results in better understanding of the utility of different sampling approaches to determine where high-risk APEC reside in the bird.

Key Words: avian; *Escherichia coli*; turkey; APEC; ecology

286 Internal organ colonization by *Salmonella* Enteritidis in experimentally infected layer pullets reared under different housing conditions.

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Invasive *Salmonella* Enteritidis infection involving the reproductive organs of laying hens can result in the

production of internally contaminated eggs, which continue to be prominent sources of disease transmission to consumers. The poultry housing environment exerts substantial influences on the dissemination and prevalence of *S. Enteritidis* in laying flocks, and the ongoing transition of the egg industry toward cage-free housing has raised new questions about the food safety ramifications. The present study assessed internal organ colonization by *S. Enteritidis* in egg-type pullets reared under different housing conditions. In one trial, pullets reared in either conventional cage or cage-free housing systems were moved at 16 wk of age into 4 isolation rooms simulating commercial cage-free barns in groups of 72 birds per room. One-third of the pullets in 2 rooms were orally inoculated with *S. Enteritidis* immediately after transfer and pullets in 2 rooms were similarly infected at 19 wk. At 6-12 d post-inoculation, all pullets were euthanized and samples of liver, spleen, and intestinal tract were removed for bacteriologic culturing. In a second trial, pullets were reared in cage free housing at either low or high bird stocking densities and transferred to the containment facility for *S. Enteritidis* infection as in the first trial. In Trial 1, *S. Enteritidis* was isolated significantly ($P < 0.05$ in Fisher's Exact Test) more frequently from spleens (55.9% v. 46.5%) and intestines (89.9% v. 82.6%) from pullets that had been reared in cages than from those reared in cage-free housing, especially when the birds were infected on the day after transfer from the rearing facility. In Trial 2, no significant differences in *S. Enteritidis* isolation frequencies from any tissue were observed between high and low density rearing groups following infection at either age, but *S. Enteritidis* was found significantly ($P < 0.05$) more often from pullets infected at 19 wk of age than at 16 wk in livers (38.9% v. 29.2%) and spleens (39.9% v. 28.1%). These results reinforce the importance of attentive pathogen risk reduction practices at this critical phase (at or just prior to sexual maturity) in the productive life of egg-laying flocks.

Key Words: Salmonella; internal organs; cage-free housing; pullet rearing

287 Immune responses, gut permeability, and cecal *Campylobacter jejuni* loads in an experimental *C. jejuni* challenge model in broilers. Emily Cason^{1*}, Walid Al Hakeem¹, Daniel Adams¹, Revathi Shanmugasundaram², Ramesh Selvaraj¹, ¹University of Georgia, Athens, Georgia, United States, ²USDA, Athens, Georgia, United States.

Campylobacter jejuni is a foodborne pathogen that colonizes the ceca and disseminates from the gut to the internal organs by compromising gut integrity. This study aimed to characterize the host immune response of broilers and broiler cecal *C. jejuni* loads to establish an experimental infection model by determining an effective dose of *C. jejuni* that will colonize the poultry ceca at 35 days of age. A total of 90 broiler chicks were randomly divided into three experimental groups in six replications. The three experimental groups were 0, 1×10^4 , and $1 \times$

10^8 CFU/bird *C. jejuni*. At 21 d of age, birds were orally inoculated with PBS (control) or 1×10^4 or 1×10^8 CFU/bird of *C. jejuni*. Feed intake and body weight were measured weekly. On 3 and 7 dpi, gut permeability was measured by FITC-dextran assay. On 3, 7, and 14 dpi, *C. jejuni* loads in cecal content were quantified by plating and cecal tonsil CD4⁺:CD8⁺ cell ratio were quantified by flow cytometry. Data were statistically analyzed using JMP Pro 15, by One Way ANOVA, followed by Tukey's test, non-parametric Kruskal-Wallis test, and by Dunn's test, when appropriate. There were no significant differences ($P > 0.05$) in BWG and FCR between the treatment groups at any of the time points studied. Birds inoculated with 1×10^4 and 1×10^8 CFU of *C. jejuni* had 2.13 and 2.22 log₁₀ at 3 dpi, 2.74 and 3.07 log₁₀ at 7 dpi, and 3.89 and 4.21 log₁₀ at 14 dpi increases in *C. jejuni* loads in the ceca compared to the control group, respectively, which had no detectable *C. jejuni* load. At 7 dpi, the CD4⁺:CD8⁺ cell ratio was ($P < 0.05$) higher in the 1×10^8 CFU of *C. jejuni* group than that in the 0 and 1×10^4 CFU/bird *C. jejuni* groups. There were no significant differences ($P > 0.05$) in serum FITC-d concentration between the treatment groups at any of the time points studied. This study found that 1×10^8 CFU/bird *C. jejuni* challenge produced greater cecal colonization and increased the CD4⁺:CD8⁺ cell ratio, compared to the Control and 1×10^4 CFU/bird *C. jejuni* groups, at 35 days old. It can be concluded that 1×10^8 CFU/bird *C. jejuni* inoculation at 21 d of age colonized the ceca at market age and induced host immune responses.

Key Words: Campylobacter jejuni; Broiler; Immunology; Microbiology; Challenge model

288 Impact of feed protein type and protease supplementation on the chicken microbiota in the context of colonization by *Campylobacter jejuni*. Sophie Chagneau¹, Marie-Lou Gaucher^{1,4}, Neda Barjesteh^{2,4}, William Thériault¹, Elizabeth Santin³, Ludovic Lahaye³, Alexandre Thibodeau^{1,4}, ¹Chaire de recherche en salubrité des viandes de l'université de Montréal, Saint-Hyacinthe, Quebec, Canada, ²Université de Montréal, Saint-Hyacinthe, Quebec, Canada, ³Jefo nutrition, Saint-Hyacinthe, Quebec, Canada, ⁴Centre de recherche en infectiologie porcine et avicole du FRQNT (CRIPA FRQNT), Saint-Hyacinthe, Quebec, Canada.

Campylobacter jejuni is one of the zoonotic pathogens the most incriminated, worldwide, for diarrheal diseases. It can reach humans via environmental exposure, direct contact with animals and through the food chain. Poultry serves as a reservoir and *C. jejuni* is found in numbers reaching up to 10^9 CFU/g in the bird's caecum. *C. jejuni* does not produce energy from sugars. Instead, it uses organic and amino acids. There are hints that this lifestyle could be exploited to facilitate *C. jejuni* control. For example, previous studies reported that changing the origin of the feed's protein modifies *C. jejuni* chicken colonization. Based on this premise, the aim of this study was to determine whether if a

change in the protein content or in protein release from chicken feed influences *C. jejuni* chicken colonization. To do so, birds were fed an animal-based diet (meat meal) or plant-based diet that was supplemented or not with a protease (0.125 g/ feed kg). Birds (n=72) were purchased from a local hatchery and housed in a level 2 biosecurity animal facility. Animals were randomly divided into 4 groups: 1) animal-based feed, 2) animal-based feed + protease, 3) plant-based feed and 4) plant-based feed + protease. Animals were orally inoculated with 2 *C. jejuni* strains (10³ CFU each) at 14 days of age. After 7 and 14 days, 9 birds per group were sacrificed; *C. jejuni* counts in the ileum, caecum and liver were performed by culture and differences between groups for each time point were analyzed using a Kruskal-Wallis test. The caecal microbiota was also analyzed by 16S sequencing. At the end of the experiment, the combination of the plant-based feed and protease gave lower *C. jejuni* caecal counts when compared to the animal-based feed (5.6 log CFU/g vs 7.6 log CFU/g) but increased ileal colonization (3.9 log CFU/g vs 2.3 log CFU/g). Extraintestinal spread was observed for the plant-based feed 7 days after inoculation (6 / 9 birds) but the addition of the protease blocked this contamination of the chicken livers (0 / 9 birds). As for the microbiota, beta diversity analysis (ADONIS test) showed that none of the conditions impacted the ileal microbiota but that both the protein type and use of the protease modified the caecal microbiota. Bacterial network analysis (sparcc correlation) also revealed that different genera were correlated with *C. jejuni* depending on the experimental group. These findings open the door to new feed-based strategies to control *C. jejuni* chicken colonization directly at the farm.

Key Words: *Campylobacter jejuni*; liver; protease; protein; control

289 Transcriptomic Analysis of *Campylobacter jejuni* and *Campylobacter coli* Survival Under Aerobic Conditions. Anand Karki¹, Mohamed Fakhr^{1*}, ¹The University of Tulsa, Tulsa, Oklahoma, United States.

Campylobacter remains a leading cause of foodborne illness around the globe. This microaerophilic microorganism encounters various stressors such as aerobic conditions and low temperature during retail meat processing and storage. In recent years, multiple studies have reported high prevalence of aerotolerant *Campylobacter* strains in various retail meats including poultry. While known to harbor genes involved in tolerance to oxidative stress, genes specific for *Campylobacter* aerotolerance during survival under aerobic conditions are yet to be identified. The objective of this study was to conduct transcriptomic analysis using RNA-Seq Illumina technology for the survival under aerobic conditions of four selected *Campylobacter jejuni* and *Campylobacter coli* strains previously isolated from mostly retail poultry meats and sequenced in our laboratory. These strains possessed variable aerotolerance levels: *C. jejuni* T1-21

(aerosensitive; nonculturable but might still be viable after 6 hours of aerobic incubation), *C. jejuni* WP2-202 (aerotolerant), *C. coli* HC2-48 (aerotolerant), and *C. coli* MG1-116 (hyper-aerotolerant). Strains were exposed to aerobic conditions in Mueller Hinton Broth media and triplicate samples were taken at 0, 0.5, 6, 12, and 24 hrs for RNA isolation. DirectZol RNA miniprep kit was used for RNA isolation and DNA was removed using the Turbo DNA free kit. cDNA libraries prepared using TruSeq stranded mRNA library preparation kit (Illumina) was sequenced in a HiSeq 4000 platform and sequence reads were analyzed using the CLC Genomic Workbench 20. Results indicated that prolonged aerobic incubation induced significant expression of various genes among all tested strains. More differentially expressed genes (DEGs) were observed in *C. jejuni* WP2-202 compared to *C. jejuni* T1-21. Likewise, more DEGs were found in *C. coli* MG1-116 compared to *C. coli* HC2-48. Despite the variation in genomic expression among *Campylobacter* strains, several genes including oxidative stress response genes and other stress response genes were found to be significantly upregulated at 12 hrs and 24 hrs compared to 0 hr for all tested strains. Higher number of genes related to inorganic iron transport and metabolism were found to be upregulated and more genes related to ribosomal proteins were downregulated for all strains at most incubation times. The majority of upregulated genes for all tested strains belonged to categories with unknown functions. In conclusion, genomic expression of *Campylobacter* survival under aerobic condition varied among the tested strains and appeared to be altered as time of incubation increases. To our knowledge, this is the first study to use RNA-Seq to investigate *Campylobacter* aerotolerance.

Key Words: *Campylobacter*; Transcriptome; RNA seq; Aerotolerance; Retail Poultry Meat

290 Modeling the Thermal Inactivation of *Salmonella* Typhimurium and the surrogate *Enterococcus faecium* in Mash Broiler Feed in A Laboratory Scale Circulated Thermal Bath. Tim Boltz^{2*}, Corey Coe¹, Rebecca Stearns¹, Cangliang Shen¹, ¹West Virginia University, Morgantown, West Virginia, United States, ²Mississippi State University, Morgantown, West Virginia, United States.

This study compares kinetic parameters of *Salmonella* and the surrogate *Enterococcus faecium* in mash broiler feed during thermal inactivation. Two-gram samples of mash broiler feed were added into a filtered sample bag and inoculated with nalidixic acid (NaL, 200 ppm) resistant *S. Typhimurium* or *E. faecium*, followed by vacuum-packaging and heating in a circulated thermal water bath at 75°, 85°, and 95°C for 0 to 180 s. Counts of bacterial survival were analyzed on tryptic soy agar and bile esculin agar plus 200 ppm of NaL. This thermal inactivation study used a 2 × 3 × 10-14 factorial structure with 2 different bacteria strains, 3 different heating temperatures, and 10-14

different heat times. Survival of *Salmonella* and the surrogate *Enterococcus* cells were analyzed using the mixed model procedure in JMP® software with individual factors of temperatures and heating time, and their interactions. Microbial data and thermal kinetic parameters [n=8, Global-Fit and United States Department of Agriculture (USDA)-Integrated-Predictive-Modeling-Program software] were analyzed by JMP software. Heating mash broiler feed at 75°, 85°, and 95°C decreased ($P < 0.05$) *Salmonella* cell counts by $> 6 \log_{10}$ CFU/g after 180, 60, and 50 s, respectively. Heating *E. faecium* in feed at 75°, 85°, and 95°C for 180, 120, and 70 s achieved reductions of 3, 6, and $> 6.5 \log_{10}$ CFU/g, respectively. D-values of linear, Weibull models, and z-value of *Salmonella* at 75°, 85°, and 95°C were 1.8 to 11.2, 4.2 to 21.8, and 28.6 s, respectively, which were lower ($P < 0.05$) than those of *E. faecium* (3.7 to 18.1, 8.5 to 34.4, and 34.1 s). Linear with Tail, Linear with Tail and Shoulder, and Weibull with Tail equations revealed that *E. faecium* were more resistant ($P < 0.05$) to heat than *Salmonella* as shown by longer “Shoulder-time” (26.5 vs 16.2 s) and greater “Tail” effect (4.4 to 4.5 vs 2.5 to 2.6 \log_{10} CFU/g. Results clearly suggested that *E. faecium* can be used as a surrogate for *Salmonella* to validate thermal inactivation during feed manufacture.

Key Words: Salmonella; Broiler feed; Enterococcus faecium; Thermal inactivation; Surrogate bacteria

291 Evaluation of curcumin and copper acetate against *Salmonella* Typhimurium infection, intestinal permeability, and cecal microbiota composition in broiler chickens. Anaisa Leyva-Diaz³, Daniel Hernandez-Patlan², Bruno Solis-Cruz², Raquel Lopez-Arellano², Bishnu Adhikari¹, Juan Latorre¹, Maria Trujillo Peralta¹, Danielle Graham¹, Roberto Senas-Cuesta¹, Aaron Forga¹, Makenly Coles¹, Billy Hargis¹, Guillermo Tellez-Isaias^{1*}, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²UNAM, Cuautitlan Izcalli, Estado de Mexico, Mexico, ³UNAM, Mexico City, Mexico.

Interest in the use of natural feed additives as an alternative to antimicrobials in the poultry industry has increased in recent years because of the risk of bacterial resistance. One of the most studied groups is polyphenolic compounds, given their advantages over other types of additives and their easy potentiation of effects when complexes are formed with metal ions. The objective of the present study was to evaluate the impact of dietary supplementation of copper acetate (CA), curcumin (CR), and their combination (CA-CR) against *Salmonella* Typhimurium (ST) colonization, intestinal permeability, and cecal microbiota composition in broiler chickens through a laboratory *Salmonella* infection model. *S. Typhimurium* recovery was determined on day 10 post-challenge by isolating *Salmonella* in homogenates of the right cecal tonsil (12 chickens per group) on Xylose Lysine Tergitol-4 (XLT-4) with novobiocin and nalidixic acid. Intestinal integrity was indirectly determined by the fluorometric

measurement of fluorescein isothiocyanate dextran (FITC-d) in serum samples from blood obtained on day 10 post-ST challenge and 16S rRNA gene-based microbiome analysis was performed using the content of the left caecal tonsil (CT) of 5 chickens per group. Data were analyzed by ANOVA. ST incidence was compared by a chi-square test of independence. The results showed that in two independent studies, all experimental treatments were able to significantly reduce the ST colonization in CT ($P < 0.0001$) compared to the positive control (PC) group. However, only CA-CR was the most effective treatment in reducing ST counts in both independent studies. Furthermore, the serum FITC-d concentration in chickens treated with CR was significantly lower when compared to PC ($P = 0.0084$), which is related to a decrease in intestinal permeability and therefore improved intestinal integrity. The effect of dietary treatments in reducing ST colonization was further supported by the Linear discriminant analysis Effect Size (LEfSe) of microbiota data, where *Salmonella* was significantly enriched in the PC group (LDA score > 2.0 and $P < 0.05$). In addition, *Coprobacillus*, *Eubacterium*, and *Clostridium* were significantly higher in the PC group compared to other treatment groups. On the contrary, *Fecalibacterium* and *Enterococcus* in CR, unknown genus of Erysipelotrichaceae at CA-CR, and unknown genus of Lachnospiraceae at CA were significantly more abundant respectively. CR treatment was the most effective treatment to reduce ST intestinal colonization and maintain better intestinal homeostasis which might be achieved through modulation of cecal microbiota.

Key Words: Salmonella Typhimurium; Broiler chickens; Curcumin; Copper acetate; Microbiota composition

292 Reduction of *Salmonella* Enteritidis in broilers fed a native *Bacillus amyloliquefaciens* in feed. Brian Dirks^{1*}, Joshua Lefler¹, James Gaffney¹, Andrew Izzo¹, Kayla Calapa¹, Jolie LoButto¹, Allison Wells¹, Mallory Embree¹, ¹Native Microbials, Norristown, Pennsylvania, United States.

Food safety, in particular *Salmonella* Enteritidis, remains a concern in broiler production. This study evaluated the effect of a native chicken-derived *Bacillus amyloliquefaciens* strain (Avius Protect™, Native Microbials, Inc.) on broilers challenged orally with nalidixic acid resistant *S. Enteritidis*. The trial consisted of 25 Cobb 500 chicks per pen with 8 pens per treatment. Treatments included a non-challenged control without the additive, a challenged control without the additive, and challenged birds with the *B. amyloliquefaciens* strain. Birds were fed additives from hatch and inoculated with *Salmonella* via oral gavage on day 7. On days 14 and 21, two birds per pen were sacrificed and cecal content were extracted for *Salmonella* enumeration and microbiome analysis. Statistical analysis was performed using a combination of one-way ANOVA, followed by Fischer’s least significant

difference (LSD) with bonferroni correction for p-value adjustments. Microbiome analysis was conducted using Usearch (v11.0) for sequence processing, OTU generation and subsequent analysis. Differential abundance analysis was performed using DeSeq2 (v3.14). Plating of cecal samples for CFU enumeration showed that *Salmonella* was reduced by 0.17 log₁₀ colony forming units (CFU)/g (P < 0.01) and 1.36 log₁₀ CFU/g (P < 0.01) in treated birds 7 and 14 days post infection, respectively. Microbiome analysis revealed several OTUs associated with a robust, healthy microbiome to be statistically increased in treated birds, such as members of the families *Lachnospiraceae* and *Ruminococcaceae*. Together these results demonstrate that this particular strain of *B. amyloliquefaciens* has a positive influence on the microbial composition of the intestinal microbiome, contributing to a reduction in the abundance of *S. Enteritidis* in the broiler intestinal tract.

Key Words: Probiotic; Salmonella; Broiler; Bacillus; Food Safety

293 *Campylobacter jejuni* cecal and carcass rinse counts from broilers fed blended organic acid, prebiotic, and probiotic additives. Matthew Bailey^{1*}, James Krehling¹, Luis Munoz¹, Cesar Escobar Lobo¹, Kaicie Chasteen¹, Kenneth Macklin¹, ¹*Auburn University, Auburn, Alabama, United States.*

Adding probiotics and organic acids to feed as a method for controlling foodborne pathogens in poultry is a growing field of study in food safety, especially in terms of less-studied pathogens such as *Campylobacter jejuni*. This study evaluated the effects of three commercial feed additives on *Campylobacter jejuni* populations in broiler chickens and on processed carcasses up to 47 days of age. These additives included a *Bacillus* spp. probiotic, a live *Saccharomyces cerevisiae* probiotic, and a commercial caprylic acid combined with the *Bacillus* probiotic. Birds were housed in floor pens with fresh pine shavings and were challenged on day 14 with approximately 1x10⁵ CFU/mL per bird of a ciprofloxacin-resistant marker strain of *C. jejuni*. Ceca samples were collected from 6 birds per pen on day 33 and 47, and processing of 6 birds per pen took place the following morning. Ceca samples and carcass rinses were evaluated for *C. jejuni* populations using plating methods. Count data was analyzed by one-way ANOVA and means were separated by Tukey's test. No significant differences in *C. jejuni* populations between treatments were detected in ceca samples or carcass rinses on day 33. On day 47, no significant differences were detected in ceca counts after performing post-hoc tests. On day 47, carcass counts were higher in the *Bacillus* probiotic treatment compared to the *S. cerevisiae* treatment, and carcass counts were higher in the caprylic acid + *Bacillus* treatment compared to the *S. cerevisiae* treatment and the control, although this may be due to processing order. Because few

significant differences in *C. jejuni* populations in ceca and carcass rinses were detected between treatments, it appears that dietary supplementation of these probiotic/organic acid products had little effect on *C. jejuni* colonization in broilers up to 47 days.

Key Words: broiler; Campylobacter; feed; probiotic; organic acid

294 Comparative study on antibacterial and growth promoting effects of copper hydroxychloride in experimentally infected broiler birds with salmonella gallinarum. Jahan Zaib¹, Asim Aslam^{1*}, Muhammad Yasin Tipu¹, Habib Rehman¹, ¹*University of Veterinary and Animal Sciences Lahore, Lahore, Punjab, Pakistan.*

The use of antimicrobial agent for prevention of bacterial disease has raised a serious question. There is extensive documentation which highlights the resistance in bacteria against multiple antibiotics. Use of alternatives of antibiotics is being compulsory in many countries of the world. Biochemically modified form of minerals like copper hydroxychloride is considered as effective supplement to fill this gap. This study was designed to evaluate the effects of copper hydroxychloride (in vitro and in vivo) on intestinal morphology and growth of poultry birds when challenged with *Salmonella gallinarum*. For in vitro study pure copper discs were applied in comparison with antibiotic discs on a plate having salmonella growth. Zone of inhibition was measured of each disc. Pure copper discs showed a significant zone of inhibition. For in vivo study, a total of 100 chicks were procured from nearest hatchery and randomly divided into 5 equal groups. Group A was negative control group which was free from any supplement and challenge. Group B was positive control and only challenge was given on day 07. Group C was given challenge as well as high dose of copper hydroxychloride @ 225ppm mixed in feed from day 1. Group D was given challenge as well as low dose of copper hydroxychloride @ 125ppm mixed in feed from day 1. Group E was given challenge as well as antibiotic zinc bacitracin @ 230g/ton of feed from day 1. Two birds were randomly selected from each group and slaughtered humanely for collection of intestinal samples on day 14 and 21 respectively. Growth rate and FCR were measured on weekly basis. Group C which was given high dose of copper hydroxychloride showed 1.5 feed conversion ratio (FCR) while rest of the other groups showed a higher values of FCR. Similarly group C showed better average weight gain than rest of the groups. Villus length, width and crypt depth were measured using Image J. software in micrometers. The results of intestinal morphometry were significant in the group which was supplemented with higher dose of copper hydroxychloride.

Key Words: intestinal morphology; Salmonella gallinarum; copper hydroxychloride ; Poultry broiler birds; FCR and weight gain

Metabolism and Nutrition, Feed Additives III

295 A precision biotic that modulates microbial metabolic pathways consistently improves the growth performance of broiler chickens: a meta-analysis. Cristiano Bortoluzzi^{1*}, Irene Vassallo¹, Alex Karagiannis-Voules¹, Jose Otavio Sorbara¹, ¹DSM Nutritional Products, Kaiseraugst, Switzerland.

The progressive reduction in the use of antibiotic growth promoters (AGP) may lead to reduced growth rate, impaired feed efficiency, and higher veterinary costs. Literature suggests that the modulation of the intestinal microbiome functions supports gut health and overall animal welfare, and consequently improves growth performance and sustainability. Additionally, it is well understood that vaccination against coccidiosis normally leads to compromised animal performance. Therefore, the objective of the present meta-analysis was to evaluate the effect of a precision biotic (PB; Symphio™, DSM Nutritional Products), that modulates core metabolic pathways of the microbiome, on the growth performance of non-vaccinated and vaccinated (coccidiosis) broiler chickens. Seventeen studies were used, comprising a total of 10,715-day-old broiler chickens (Ross 308, Ross 708, Cobb 500, Arbor Acres, Yellow Feather broilers), fed diets commonly used worldwide, and raised from 0 to 34, 35, 41, 42, 46, 49, or 80 days of age. The experimental groups were control diets (no supplementation) or supplementation of PB ranging from 0.9-1.1 kg/ton. Feed intake (FI) and body weight (BW) were measured at the end of each study. Corrected feed conversion ratio (cFCR) was calculated by considering the weight of dead birds and corrected with a common body weight. The overall effect of PB was analyzed, as well as the effect in non-vaccinated and vaccinated birds. A random-effect model was fitted to the data. The amount of heterogeneity was estimated by using restricted maximum-likelihood estimator. Considering the overall growth performance of the broilers, the PB improved cFCR by 3.64 points ($P = 0.0004$), and BW by 30 g ($P = 0.01$). The PB supplementation improved the cFCR of vaccinated birds by 5.77 points (9 studies; $P = 0.002$), and by 2.31 points (8 studies; $P = 0.006$) in non-vaccinated birds. Additionally, PB improved the BW of vaccinated birds by 66 g (9 studies; $P = 0.007$), and by 11 grams (8 studies; $P = 0.23$) in non-vaccinated birds. These results show that this novel PB, by modulating microbial metabolic pathways, consistently improves the growth performance of broiler chickens, being these benefits more evident in vaccinated broilers.

Key Words: chickens; microbiome; precision biotics; core metabolic pathways

296 Effect of a phytogenic feed additive in broilers under an early coccidiosis challenge. Marcos Rostagno^{1*}, William Varner¹, Randolph Mitchell², ¹Phytobiotics North America, LLC, Cary, North Carolina, United States, ²Perdue Foods, LLC, Salisbury, Maryland, United States.

The objective of this study was to evaluate different programs of a phytogenic feed additive (Sangrovit®, Phytobiotics North America, LLC) in broilers subjected to an early coccidiosis challenge. A total of 2,760 one-day-old male chicks (ROSS 708) were divided into 60 floor-pens (12 pens/treatment with 46 birds/pen) allocated to 5 treatments: 1) Negative control (no feed additive); 2) Phytogenic feed additive (PFA) at 60-60-60 ppm; 3) PFA at 90-60-60 ppm; 4) PFA at 60-90-60 ppm; and 5) PFA at 60-60-90 ppm, based on the 3-phase feeding program utilized (Starter, Grower, Finisher), using corn-soybean based diets fed *ad libitum* for 6 weeks (42 days). All birds were challenged with 5X the recommended dosage of a commercially available live coccidiosis vaccine at the hatchery and reared on re-used litter. Body weight and feed intake data were collected at 14d, 28d and 42d, and feed conversion ratio (FCR) calculated for the same time-points. Data was analyzed using one-way ANOVA, followed by Tukey's test to identify differences between means ($p < 0.05$). There was no difference in mortality between treatments, throughout the study period with overall mortality of 1.41% at 14d, 2.58% at 28d, and 3.22% at 42d. Body weight did not differ between treatments at 14d, while significant differences occurred at 28d and 42d ($p = 0.001$) with the PFA 60-60-60 program being consistently superior. There was no difference between treatments in feed intake at 14d and 28d, although the total feed intake at 42d was significantly higher for the PFA 60-60-60 program ($p = 0.019$). There was no difference in FCR at 14d and 42d, but at 28d a significant difference occurred with best results observed in the PFA 60-60-60, 90-60-60 and 60-60-90 programs ($p = 0.003$). During the grower period (14-28d), inclusion of the PFA improved body weight gain and feed efficiency, resulting in birds with a 0.125 lbs. body weight advantage and a mortality-adjusted FCR advantage of 4.4 points ($p = 0.0001$ for both parameters). At the end of the study (42d), inclusion of the PFA improved body weight by 0.25 lbs. ($p = 0.001$). In conclusion, this study demonstrates that the phytogenic feed additive, Sangrovit®, results in significant growth and efficiency improvements in broilers subjected to an early high coccidiosis challenge, with no clear difference observed between the evaluated programs.

Key Words: Broilers; Coccidiosis; Phytogenic; Growth performance; Feed additive

297 Evaluation of sodium bisulfate on the performance of turkeys with coccidiosis vaccination. Katherine Cupo¹, Zohreh Mehdipour^{1*}, Juan C. Suarez², Kelli Jones³, Robert Beckstead³, Chongxiao (Sean) Chen¹, ¹North Carolina State University, Raleigh, North Carolina, United States, ²Jones-Hamilton Co. Agricultural & Animal Nutrition, Walbridge, Ohio, United States, ³Ceva, Lenexa, Kansas, United States.

Coccidiosis is a common protozoan challenge in turkey production. It reduces feed intake and weight gain as well as causes mortality in commercial turkey flocks. Combination of feed additives that improve gut health and bird performance with the coccidia vaccine may be a practical approach to limit the negative effects of the vaccine while still boosting the young birds' immunity to subsequent infection with coccidia oocysts. This study was performed to investigate the effects of dietary supplementation of animal feed grade sodium bisulfate (AFG) on the performance of turkeys given a 2x dose of a coccidiosis vaccine. 960 one-day-old male Nicholas Select poults were randomly divided into 6 experimental groups with 8 replicates in a 2×3 factorial arrangement with non-vaccinated or vaccinated treatment (oral inoculation of 2x IMMUCOX[®]T at day 0) and dietary supplementation of 0% (control), 0.2%, or 0.4% AFG. Oocyst counting was performed on day 7 and 14 to confirm parasite cycling and vaccine viability. Body weight (BW), feed intake (FI), and feed conversion ratio (FCR) were evaluated at 0, 35 and 56 days. On day 56, all birds were weighed individually to determine uniformity using standard deviation. Data were analyzed by MIXED procedure in SAS v9.4 with the main fixed effects of Feed Additive, Vaccination Status, Feed Additive x Vaccination Status and Room Block. Day 0 body weight as covariate. Significance was determined when $P < 0.05$. Means were separated using the PDIF option. The results showed that oocyst per gram of feces (OPG) were not significantly altered between treatments. From the interaction effects, the 2x coccidiosis vaccine caused a failure to negatively impacted gain weight at 35 days of age ($P < 0.0001$), which was recovered at 56 days of age. Vaccinated birds given 0.4% AFG achieved higher BW (1738g) at 35 days ($P = 0.0055$) compared the rest of treatments (1572 to 1653 g). Furthermore, from the main effects, birds given 0.4% AFG had lower FCR (1.583) from 0-35 days compare to control (1.645) ($P = 0.0460$), and both 0.2% and 0.4% AFG groups had lower FCR compared to birds given the control diet from 0-56 days ($P = 0.0100$). Body weight uniformity was not significantly altered between treatment groups or vaccinated groups at 56 days (from 9.58 to 11.18). The results suggested that the combination of dietary supplementation of animal feed-grade sodium bisulfate and coccidiosis vaccination could be an effective measure to alleviate the early performance losses caused by coccidiosis vaccination in turkeys.

Key Words: Performance; Coccidiosis; Turkey; Vaccination; Sodium bisulfate

298 The effect dactitic tuff breccia (Azomite) and its fractions in a corn, soybean, and dried distillers grains with solubles-based diet on pellet mill production rate and pellet quality. Tim Boltz^{1*}, Jon Ferrel⁴, Fernanda Castro⁴, Kristina Bowen², Elizabeth Lynch², Victoria Ayres³, Joseph Moritz², ¹Mississippi State University, Morgantown, West Virginia, United States, ²West Virginia University, Morgantown, West Virginia, United States, ³Tennessee

Technological University, Cookeville, Tennessee, United States, ⁴AZOMITE, Nephi, Utah, United States.

The use of inorganic phosphate sources (IPS) has been in decline, prompting feed manufacturers to utilize ingredients to maintain production rate benefits from pellet die channel scouring and lubrication. Past research has shown that Azomite (AZM) can be supplemented in diets to improve production rate, reduce motor load, and decrease electrical use of the pellet mill. AZM is composed of a fine and coarse fraction, but the mechanism of how each enacts its effect at the pellet die has not yet been determined. The objective was to assess the response of AZM and its two fractions in a corn, soybean, and dried distillers grains with solubles (DDGS)-based diet on pellet mill production rate, hot pellet temperature, and pellet quality. One basal diet (control) was either supplemented with AZM or each of its corresponding fractions, manufactured in a Latin Square with a crossover design across 4 days. Data were analyzed in a mixed-model analysis using the GLIMMIX procedure of SAS (Release 9.4, SAS Inst. Inc., Cary, NC) with alpha set at $P \leq 0.05$. Average ambient temperature and humidity were 27°C and 67%, respectively. In support of previous research, the inclusion of AZM increased production rate by 7% when added to the basal diet (0.999 vs. 0.935 MT/h; AZM vs. Control, respectively; $P = 0.005$). The combination of both fractions likely contributes both a lubricating and pellet die scouring effects, increasing production rate more than either single fraction (0.999 vs. 0.953; AZM vs. Fine; $P = 0.063$; 0.999 vs. 0.951; AZM vs. Coarse; $P = 0.045$; 0.953 vs. 0.951; Fine vs. Coarse; $P = 0.999$). Inclusion of AZM or its fractions did not significantly impact pellet temperature (83.79 vs. 83.73; Control vs. AZM; $P = 0.989$; 83.79 vs. 83.65; Control vs. Fine; $P = 0.892$; 83.79 vs. 83.88; Control vs. Coarse; 0.977). The addition of the fine fraction increased pellet quality (90.32% vs. 87.85%; Fine vs. Control; 90.32% vs. 88.31%; Fine vs. Coarse; 90.32% vs. 88.82%; Fine vs. AZM, respectively; $P < 0.001$), likely due to clay-like particles retaining more moisture and acting as a pellet binder. These results show that AZM's fractions work synergistically to improve the production rate.

Key Words: Feed Manufacture; Inorganic Phosphate Source; Production Rate; Throughput; Tuff Breccia

299 Influence of the inclusion of a postbiotic from *Aspergillus oryzae* on productive performance and egg quality of hens from 18 to 41 weeks of age. A.F. de Juan^{1*}, J. Ben Mabrouk¹, C. Ocasio-Vega², N. Corrales¹, Gonzalo Mateos¹, ¹Universidad Politécnica de Madrid, Madrid, Spain, ²BioZyme Incorporated, St. Joseph, United States.

The effects of the inclusion in the diet of a postbiotic derived from *Aspergillus oryzae* (AO) on performance and egg quality traits was studied in of Lohmann Brown Classic hens from 18 to 41 wk of age. The postbiotic was produced via submerged fermentation with AO under controlled

conditions (BioZyme, Inc., St Joseph, MO). The experimental design was completely randomized with 2 diets that consisted in a control diet with 2,750 kcal AMEn/kg and 16.9% of CP (Control) and the same diet supplemented with 50 g of the postbiotic per ton of feed (AOSO). Each treatment was replicated 18 times and the experimental unit was a cage with 6 hens. The experiment lasted 24 wk (6 periods of 4 wk each). Egg production and number of broken and shell-less eggs were controlled daily and feed disappearance and egg weight were measured weekly. Any mortality was weighed as produced. The proportion of albumen, yolk, and shell of the eggs, shell quality traits (weight, strength, and thickness), and Haugh units, were measured in all eggs produced the last 2 d of each of the 6 experimental periods. BW of the hens was measured at the start of the experiment and at the end of each of the 6 experimental periods. The experiment consisted of 2 phases: pre-peak (18 to 25 wk of age) and peak production (26 to 41 wk of age). Data were analyzed as a completely randomized design with diet as main effect using the GLM procedure of SAS. During the pre-peak phase, no differences were observed for any of the production or egg quality traits studied. In the peak production phase however, egg mass was greater in hens fed AOSO than in hens fed the control diet (64.4 vs. 62.7 g/d; $P < 0.05$). Also in this phase, AOSO supplementation increased egg production (98.2 vs. 96.6%) and egg weight (65.6 vs. 64.9 g), and improved FCR (1.84 vs. 1.90) but the differences were not significant. Diet did not affect any of the egg quality traits studied, at any time. For the whole experiment (18 to 41 wk), AOSO supplementation improved all major production traits studied, included egg mass (50.6 vs. 49.7 g/d) and FCR (2.27 vs. 2.32), but the differences were not significant. The benefits of AOSO supplementation on all the production variables studied were observed for each period of the peak production phase. Probably, the high level of production of the control hens reported (over 49.5 g/d), precluded the possibilities of detecting significant differences between both groups.

Key Words: egg quality; laying hen; hen performance; postbiotic; *Aspergillus oryzae*

300 Immunity enhancing dried egg product improves broiler growth performance via preserving intestinal health. Daniel Adams², Pradyut Paul¹, Kimberly Livingston^{3*} ¹Optum Immunity, Waterloo, Wisconsin, United States, ²North Carolina State University, Raleigh, North Carolina, United States, ³Optum Immunity, Siler City, North Carolina, United States.

Enteric pathogens (such as *Eimeria*, *Clostridium* and *Salmonella*) adversely affect intestinal health in chickens, leading to major financial losses each year to the poultry industry by impacting feed conversion and weight gain. An ever-growing consumer concern against antibiotics and other commercial drugs in the poultry industry and the demand for antibiotic free (ABF) and No Antibiotics Ever

(NAE) poultry severely limits the tools available to treat enteric infections. Widely used coccidiostats, such as nicarbazin are still effective at controlling coccidiosis, however, will eventually lead to resistant pathogen strains and may alter the animal's physiology which may impact survivability. Overall, the need for more natural alternatives to chemical treatments is increasing. Egg products are highly nutritious, containing large amounts of protein, vitamins, minerals, and lipids. Additionally, eggs contain many protective compounds capable of modulating an animals' immune response. Here, we report the impact of an enhanced Dry Egg Product (eDEP) on broiler performance and survivability in comparison to Nicarbazin when birds are raised on used litter that exposes them to natural intestinal insults. Male Ross 708 chicks were randomly distributed into one of three dietary treatment groups: 1. Standard US control, 2. DEP, 3. Nicarbazin (Nicarb) with 24 reps per treatment. Birds were raised in floor pens on used litter for 42 days. eDEP was fed through 28 days while Nicarb was fed through 37 days. Performance parameters including feed consumption, average body weight, average daily gain, and feed conversion ratio were determined. Intestinal lesions were visually analyzed on a scale of 0 to 4 from 2 birds per pen at day 14 using methods pioneered by Johnson and Reid. Fecal samples were taken during each study to obtain oocysts per gram of fecal material using the McMaster egg counting technique. Data were analyzed using one-way ANOVA in JMP Pro 14, and a $P < 0.05$ was considered significant. No differences were noted between the eDEP and nicarbazin treated group in terms of oocysts per gram. However, eDEP showed significant improvements in survivability ($P < 0.05$) when compared to the nicarbazin group. Additionally, birds had improved FCR and Average Bodyweight throughout the study when compared to the nicarbazin treated group. In conclusion, eDEP improved broiler performance when fed during the starter and grower period, and may be a safer, more natural alternative to some commercial coccidiostats.

Key Words: Broilers; intestinal health; Coccidiosis; Nicarbazin; Egg Product

301 Evaluation of guanidinoacetic acid as an energy source in the diets of modern broilers. EAF Butolo², LL Costa³, MR Silva³, RJP Felicio², Arele Calderano⁴, Marlene Schmidt⁵, Jose Rivera Ulloa^{1*}, ¹Alzchem GmbH, Florianopolis, Santa Catarina, Brazil, ²Integral Agroindustrial LTDA, Fortaleza, Brazil, ³Atlantica Alimentos, Fortaleza, Brazil, ⁴Federal University of Viçosa, Viçosa, Brazil, ⁵NutriQuest, São Paulo, Brazil.

Due to rapid growth, modern broilers have a high-energy demand. This favors the use of oils and fats in broiler feed (Macari et al., 2002). According to Pacheco (2014), dietary energy donors are one of the most expensive components in diets. Creatine, and phosphocreatine plays a crucial role in cellular energy metabolism by deliver the phosphate group for ATP synthesis. By adding guanidinoacetic acid (GAA),

endogenous Creatine synthesis is increased and hence, energy metabolism is improved. This allows assigning an energy sparing matrix value to GAA. The objective of this experiment was to determine whether guanidinoacetic acid (GAA) in the diet of broilers can compensate for a reduction of 100 kcal of ME/kg of feed. The study was carried out at the experimental aviary of granja regina, located in Brazil. A total of 1200 Ross Ap95 one-day-old male chickens, with an initial body weight of 39.2 ± 1.05 were allotted to three treatments, with eight replicates of 50 chickens each. The experimental design was completely randomized. The trial comprised five feeding phases of one week each. The treatments were: T1: Positive control (PC) with these levels of metabolizable energy (kcal/kg of feed): Pre-initial: 2900; Initial: 3150; Fattening 1: 3200; Fattening 2: 3250; Final: 3250; T2: Negative control (NC) (-100 kcal in each phase, as compared to PC) and T3: NC + 600 g/ton of GAA (Creamino®). The body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), daily weight gain (DWG) and productive efficiency index (PEI) were determined. The data were subject to analysis of variance; when significant differences were found ($P < 0.05$), the means were compared by the Dunnett test, using the SAS statistical package (SAS INSTITUTE, 1999). During the whole period, reduction in dietary energy (NC) led to worse performance (BWG, FCR, DWG and PEI) when compared to the PC ($p < 0.05$). When GAA was added to the NC (NC + GAA), performance was improved and reached same levels to the PC group. The addition of GAA at 0.06% (600 g/t feed) compensated a dietary energy reduction of 100 kcal ME/kg feed without affecting performance of modern broilers. This implicates that expensive energy donors in feed can be reduced and hence, feed costs can be lowered.

Key Words: Creatine; Energy matrix; Metabolizable energy; Phosphocreatine; Poultry nutrition

302 Dietary phytogetic inclusion level effects on egg production, egg quality and expression of ovarian cytoprotective genes in laying hens. Ioannis Brouklogiannis¹, Evangelos Anagnostopoulos¹, Vasileios Paraskeuas¹, Eirini Griela¹, Andreas Kern², Konstantinos Mountzouris^{1*}, ¹*Agricultural University of Athens, Athens, Greece*, ²*ANCO Animal Nutrition Competence GmbH, Sankt Poelten, Austria*.

A 12-week study was conducted to investigate the effects of a phytogetic feed additive (PFA) inclusion level on egg production, egg quality and the expression of genes relevant for signaling inflammation (nuclear factor-kappa B; NF- κ B), detoxification (aryl hydrocarbon receptor; AhR) and antioxidant capacity (Nuclear factor erythroid 2-related factor 2; Nrf2) in the ovaries of laying hens. The PFA consisted of ginger, lemon balm, oregano, and thyme substances (Anco FIT-Poultry). Laying hens ($n=385$; 20-wk-old; Hy-Line Brown) were randomly allotted to 5 dietary treatments with 7 replicates of 11 hens each. Dietary

treatments included: a basal diet without PFA addition (CON), and basal diet supplemented with PFA at 500 (P500), 750 (P750), 1000 (P1000) and 1500 (P1500) mg/kg diet. Egg production and quality parameters were determined weekly until the 32nd week of layers age and reported as overall. At the end of the experiment, a layer from each replicate was randomly selected and euthanized and the ovaries were removed and stored deep frozen until gene expression analysis. The experimental data were analyzed by ANOVA procedure and statistical significance was determined at $P < 0.05$. Biological response patterns with respect to the PFA inclusion level was studied using polynomial contrasts. Results revealed that increasing PFA inclusion, enhanced linearly and quadratically egg laying rate, with P1000 birds being higher ($P < 0.001$) compared to CON. Incremental levels of PFA linearly and quadratically increased albumen height and Haugh unit, with P750 and P1000 being higher ($P < 0.01$) than CON. Shell mass increased quadratically with increasing PFA inclusion with peak at P1000 ($P < 0.05$). In the ovaries, expression of the majority (13 out of 15) of NF- κ B pathway genes assessed were down regulated ($P < 0.05$) mainly at P1000 and P1500 treatments. From the AhR pathway genes, the expression of Cytochrome P450-B1 (CYP1B1) was linearly ($P < 0.01$) and significantly ($P < 0.01$) reduced with increasing PFA level. In addition, PFA related cytoprotective potential was demonstrated via beneficial changes seen for the majority (9 out of 11) of the Nrf2-pathway genes assessed with the P1000 displaying most significant differences from CON. Conclusively, new data highlighted beneficial cytoprotective effects of PFA inclusion on layer ovaries and document further egg production and quality improvements, with the 1000 mg PFA/kg diet being the most prominent inclusion level.

Key Words: egg quality; performance; ovaries; nutrigenomics; phyto-genics

303 Production performance and gut cytoprotective response in laying hens fed with different phytogetic levels. Evangelos Anagnostopoulos¹, Ioannis Brouklogiannis¹, Vasileios Paraskeuas¹, Eirini Griela¹, Andreas Kern², Konstantinos Mountzouris^{1*}, ¹*Agricultural University of Athens, Athens, Greece*, ²*ANCO Animal Nutrition Competence GmbH, Sankt Poelten, Austria*.

The aim of this work was to evaluate the effects of 5 dietary inclusion levels of a phytogetic feed additive (PFA) on production performance and on underlying inflammatory, detoxification, and antioxidant molecular mechanisms in the duodenum and the ceca of laying hens. The PFA was based on ginger, lemon balm, oregano, and thyme substances (Anco FIT-Poultry). A total of 385 20 wk-old Hy-line Brown layers were randomly assigned into 5 dietary treatments, with 7 replicates of 11 hens each, for a 12-week feeding trial. Experimental treatments received a corn-soybean meal basal diets with no PFA (CON) or supplementation with PFA at 500 (P500), 750 (P750), 1000

(P1000) and 1500 mg/kg diet (P1500), respectively. Layer egg mass, feed intake and feed conversion ratio were determined weekly and reported here on an overall performance basis. Duodenal and cecal intestinal samples from 32-wk-old layers were collected and stored deep frozen, until gene expression analysis with qPCR. Data were analyzed by ANOVA and statistical significance was determined at $P < 0.05$. Linear and quadratic patterns of biological responses to PFA inclusion levels were studied via polynomial contrasts analysis. Egg mass was significantly increased ($P < 0.01$) with differences up to 4% in the P1000 group, compared to CON. At duodenum, increasing dietary PFA inclusion level down regulated ($P < 0.05$) the expression of most of inflammatory and detoxifying genes involved in nuclear factor-kappa B (NF- κ B) and aryl hydrocarbon receptor (AhR) signaling pathways, respectively. On the contrary, most of the antioxidant genes (8 out of 11) implicated in nuclear factor erythroid 2-related factor 2 (Nrf2) pathway were increased ($P < 0.05$) with increasing PFA level, with P1000 being predominately higher than CON. Similarly, at cecal level most of the genes related to NF- κ B (12 out of 15) and AhR (3 out of 6) pathway were down regulated ($P < 0.05$), while those involved in the Nrf2 (4 out of 11) pathway were up-regulated ($P < 0.05$) with increasing PFA inclusion level with the higher expression levels obtained in treatments P1000 and P1500. In conclusion, our research data demonstrate that PFA inclusion downregulated layer inflammatory and detoxification gene expression responses, whilst increasing the expression of antioxidant response genes along with an overall layer performance enhancement, with P1000 displaying optimal benefits.

Key Words: laying hens; performance; phytogetic; gut function; nutrigenomics

304 Effects of various doses and combinations of phytonutrient and tributyrin on the performance of 55 to 85-week-old Hy-Line W36 laying hens. Mike Persia^{1*}, E. Nicole Thetga¹, Nathaniel Barrett¹, Brian Glover², Jose Charal³, Milan Hruby², ¹*Virginia Tech, Blacksburg, Virginia, United States*, ²*ADM, Morgantown, West Virginia, United States*, ³*ADM, Auburn, Georgia, United States*.

An experiment was conducted to determine the effects of two feed additives and their combinations on late first-cycle laying hen performance and egg quality. The eight

treatments were generated using a corn-soybean meal-dried distillers grains with solubles-poultry biproduct meal basal diet with the addition of feed additives on top. Treatments included the control diet (Con); Con + 50 g/MT of Nextend (ADM, Decatur, IL, USA) Half (NH – phytonutrient); Con + 100 g/MT of Nextend Full (NF); Con + 250 g/MT Daaforce 100 (ADM, Decatur, IL, USA) Half (DH - tributyrin); Con + 500 g/MT Daaforce 100 Full (DF); Con + NH + DF (NHDH); Con + NF + DH (NFDH); Con + NF + DF (NFDF). Each treatment was fed to 12 experimental units of 3 Hy-Line W-36 laying hens from 55 to 85 weeks of age. All hens were housed in battery cages (464.5 sq cm) and given ad libitum access to water and fed approximately 95 to 97 g/d. Repeated measures were used over time to increase replication. If ANOVA differences were noted ($P \leq 0.05$), Fishers LSD were used to separate LS means. No interactions between treatment and time were noted indicating all responses were consistent over time. Performance parameters were different ($P \leq 0.05$) with the exception of feed intake as that was controlled. Hen house (HHEP) and hen day egg production (HDEP) were generally not improved over the Con (75.6 and 76.7% respectively) with feed additive treatment ($P > 0.05$) with the exception of HHEP for hens fed DF (79.5%). The NF (63.1 g) and NFDF (63.1 g) increased egg weights in comparison to Con fed hens (61.7 g) with other treatments intermediate ($P \leq 0.05$). Egg mass was increased over the Con fed birds (47.3 g/d) with the addition of NF (50.2 g/d: $P \leq 0.05$) with DH, DF, NFDF resulting in intermediate responses. Feed efficiency was increased by NF (524 g/kg), DF (526 g/kg), NFDH (517 g/kg) in comparison to Con fed hens (494 g/kg) with DH intermediate ($P \leq 0.05$). Yolk color, Haugh units, yolk weight, egg shell breaking and egg specific gravity were not different than Con fed hens ($P > 0.05$). Hens fed DF resulted in increased albumen weight in comparison to Con fed hens, and hens fed NF and NFDF resulted in increased shell weights in comparison to Con fed hens. Overall, the full doses of Nextend and DaaForce 100 increased 55 to 80 wk laying hen feed efficiency with mixed results from the combination of these products.

Key Words: laying hen; performance; phytonutrient; tributyrin

Poster Presentations

305P A pecking block preference test on furnished-caged Lohmann Lite hens. Taiwo Makinde^{1*}, Stephanie Cottee², Bertrand Medina², Deborah Adewole¹, ¹*Dalhousie University, Truro, Nova Scotia, Canada*, ²*Probiotech International Inc., St-Hyacinthe, Quebec, Canada*.

Pecking is a natural behavior of hens associated with exploratory and foraging activities. Commercially available pecking blocks are designed to enrich the environment and reduce the incidence of harmful (redirected) behaviors such as feather pecking and cannibalism. It is accepted that hens' desire to peck is determined by various attributes (such as shape, color, size, taste, texture, or nutritional composition) of the available pecking block. While some studies have reported encouraging results with reductions in feather pecking and indications of improved overall welfare in alternative housing systems (aviaries, on-floor, and outdoor), no study has investigated the preference of laying hens for pecking blocks in furnished cages. Thus, we investigated the free choice of Lohmann Lite hens for several options of pecking blocks in a furnished cage system. A total of 280 Lohmann Lite hens (33-week-old) were randomly allocated to 8 furnished cages of 35 hens/cage for 14 days. Three well-compacted, brown-colored commercial pecking blocks were obtained from three different manufacturers (A, B, and C) and randomly assigned to the cages. Blocks A and B are rectangular-shaped, while block C is cylindrical-shaped. The average initial weights of blocks A, B, and C were 2,757.25g, 2,732g, and 1,243.75g, respectively. The pecking blocks were attached to one end of each cage using a chain in an A-B-C design. While the location of the pecking block triad was uniform in all cages, the individual block-type positions were switched within the triad at the end of day 7 to ensure that usage was not affected by position. Block weights were taken on days 0, 7, and 14, and the weight difference was used in calculating the total amount pecked/bird. Data were analyzed using Minitab 19.0, the difference in means was analyzed using a one-way ANOVA, and mean separation was conducted using Tukey's HSD. The mean gram consumption/bird of block A was significantly higher ($P<0.05$) than blocks B and C (9.643g, 3.832g, and 0.232g, respectively) among the three blocks tested, showing that hens preferentially used block A more than others. This preference test shows that hens peck at different block options at different rates when offered the choice of more than one option. These results demonstrate that different ingredient compositions and textures may influence birds' long-lasting usage of pecking block enrichments.

Key Words: laying hens; furnished cages; preference test; pecking block

306P Preventing feather pecking and cannibalism for W-36 pullets in cage-free houses. Ramesh Bist^{1*}, Lilong

Chai¹, Sachin Subedi¹, Xiao Yang¹, ¹*University of Georgia, Athens, Georgia, United States*.

Egg production is shifting from conventional cage to cage-free (CF) due to concerns of animal welfare in the US. While CF housing system provides birds more space to perform their natural behaviors, a particular challenge is severe feather pecking (SFP, i.e., damage with blood), which is one of primary reasons why CF housing has higher mortality and injury rates than conventional cage system. Addressing this issue will be helpful for protecting animal welfare and reduce the economic loss on CF egg farms. The primary objectives of this study were to 1) examine the effects of pullets' age on SFP and identify the best management strategies (e.g., pecking block, isolation chamber, and chemical body lotion) to prevent cannibalism and early death of pullets. A total of 800 Hy-line W-36 commercial day-old chicks were randomly allocated into four identical rooms with same feeding/drinking system, temperature/ventilation control, and light conditions as recommended by industry standard. The daily production information, SFP, and mortality were recorded and analyzed from 1 to 16 weeks of age (WOA). Pecking block has been used in commercial poultry houses for preventing pecking. In this study, color, i.e., colored block (e.g., red, green, & blue) and pecking block (Alltech pecking block), were introduced at 14 WOA and 16 WOA, respectively. Effect of block on pecking prevention were monitored with imaging system (six cameras each room) manually every day. After observing any pecking damages, injury birds were isolated in a specially designed isolation chamber (IC, L:8×W:4×H:2.5 ft) in each room for a week or longer until the SFP is not visible. A lotion product (Rooster Booster Pick-No-More lotion, Tractor Supply Co., GA.) was sprayed on the pecked places for preventing the area from further pecking. The effect of pecking blocks, IC, and lotion on SFP were analyzed using one-way ANOVA by JMP Pro-16. The mean for weekly apparent FP data was separated using Tukey HSD methods, while the mean for before and after applying lotion inside IC was compared using the student t-test. The effect was considered significant at $p<0.05$. The results show that the FP was significantly increased as the bird's age increased ($p<0.01$). Isolating pecked birds into IC had stopped SFP for a while but could not control further FP, so applying lotion had resulted in a significant decrease in SFP ($p<0.01$) and stopped cannibalism & death rates ($p<0.05$). Although birds like pecking blocks, but the FP rates were not reduced over time. Overall, IC with lotion treatment on pecked birds reduced the mortality.

Key Words: feather pecking; cannibalism; Animal welfare; cage-free housing

307P Vegetable-poultry-cover crop system vs conventional: Year 1 Freedom Ranger performance, bone quality, and welfare outcomes in a multi-year

study. Kayla Elmore^{1*}, Anne Carey¹, Ajay Nair¹, Elizabeth Bobeck¹, ¹*Iowa State University, Ames, Iowa, United States.*

The implementation of livestock into cover crop systems prior to vegetable rotation is a sustainable practice that has been shown to improve soil health and system diversity. Producers who choose to integrate cover crops may do so with various crops, including those that may be sold directly to the consumer. Relatively little is known about performance changes when integrating poultry into the crop rotation cycle, and even less is reported directly comparing pasture vs. indoor poultry. The objective was to compare performance and physiological outcomes of Freedom Ranger broilers raised in a pasture vs. conventional indoor setting. Two replicates of 176 Freedom Ranger broilers were started in brooders for 21d. The first replicate occurred from spring to summer (Group 1) and the second from summer to fall (Group 2). On d21, birds were randomly allocated to 2 treatments, indoor floor pens (IN) and outdoor chicken tractors (OUT; 5 ft x 10 ft plots), for a 6-week grow-out cycle. OUT birds were raised on spinach plots during the first replicate and on cowpea and grass teff during the second replicate. Performance and welfare measures were recorded weekly. At d64, 40 birds per treatment were euthanized for tibia collection and bone quality analysis using Dual-energy X-ray Absorptiometry (DXA, Hologic). Performance and DXA data were analyzed using Proc Mixed. Lameness data were analyzed using the Proc Freq (SAS version 9.4) with significance at $P \leq 0.05$. No significant differences were detected in body weight, feed intake, gain, or FCR during the starter period (d21-35) for either housing method in group 1. The only performance difference observed in group 1 was on d63, where IN had a lower FCR than OUT ($P=0.0001$). Group 2 OUT had an increased average feed intake by 12.1% during the grower period (d35-49, $P=0.0031$), 21.6% in finisher (d49-63, $P=0.0002$), and overall (d21-63, 13.2%, $P=0.0018$) compared to IN. This increased feed intake was reflected in increased BW by 11.2% ($P=0.0097$) and weight gain and average daily gain in d63 by 25.9% ($P=0.0240$). Breast and footpad conditions were not affected by treatment in either replicate group ($P>0.05$), but there was a 4.2% higher prevalence of lameness in group 1 OUT birds. Additionally, the group 1 OUT birds had 23.5% more bone mineral content and 8.3% more bone mineral density than IN birds ($P=0.0147$ and 0.0037). However, there were no differences in bone area, mineral content, or density among treatments in group 2. In conclusion, housing type did not have a significant effect on footpad or breast conditions of slow-growing broilers, and the OUT housing setting did not negatively impact performance but may positively impact bone mineral content and density.

Key Words: bone quality; broiler; performance; welfare; pasture-raised

308P Influencing satiety in the pursuit of welfare: Effect of dietary fatty acid profile and probiotic delivery

method on 3-week bodyweight and daily feed intake of 2 strains of broiler breeder pullets. Nikolas Faust^{1*}, Rosemary Walzem¹, Sean Davies³, Kristin Moncada^{2,1} Tara Price¹, ¹*Texas A&M University, Bryan, Texas, United States*, ²*Pilgrims, Greeley, Colorado, United States*, ³*Vanderbilt University, Nashville, Tennessee, United States.*

Breeder pullets begin feed restriction (FR) ~ 3 weeks post-hatch. Despite effectively increasing hen livability and chick yield, FR is a welfare issue. We seek to identify methods to mitigate hunger stress, and identified a probiotic able to reduce voluntary feed intake (FI) in pullets when delivered by daily gavage (+G). Because gavage constitutes a daily handling stress, efficacy of probiotic delivery via water cup (+WC) was tested. 200-day old breeder pullets from two commercial strains were randomly assigned to one of 40 groups of similar starting bodyweights (BW) (Breed 1: 40 ± 3 g Breed 2: 41 ± 3 g). Five groups of each breed ($n=5$ /group) were randomly assigned to one of 4 treatments. Separate battery compartments within 6-tier, battery cages (pen) housed each group. Treatments were 1) Usual diet (US), 2) Mediterranean diet (MED), 3) MED + G, 4) MED + WC. Probiotic dose provided 1×10^8 CFU *e. coli* Nissle 1917/mL/bird/day. Both diets met breeder recommendations, but MED was oleic acid rich compared to US. We report 21-day BWs ($n = 50$ birds/treatment/breed) and feed intake ($n=10$ pens/treatment/breed) from day 14-21. Two-way ANOVA (JMP) tested the main effects of diet and handling; Tukey-Kramer was used for post-hoc means testing. Values are means \pm SEM and considered significantly different at $p \leq 0.05$. Breeder standard 21 d BW: Breed 1, 308g; Breed 2, 405g. 21-day BW(g) of breed 1 and 2 for US, MED, MED+gavage, and MED+WC were found to be 312 ± 59^C , 309 ± 60^C , 222 ± 45^D , 253 ± 47^D and 511 ± 114^A , 403 ± 48^B , 374 ± 60^B , 383 ± 51^B , respectively. Feed intake (g/b/d on wk. 3) of breed 1 and 2 for US, MED, MED+gavage, and MED+WC were found to be 33 ± 5^{BC} , 31 ± 8^C , 24 ± 11^D , 23 ± 8^D and 42 ± 12^A , 39 ± 4^{AB} , 35 ± 6^{ABC} , 36 ± 6^{ABC} respectively. Oleate rich MED diet, did not affect BW of Breed 1, but in combination with probiotic delivered by either +G or +WC reduced BW by 29%-19%, respectively, compared to the average BW of US and MED (310.7g). Handling stress (+G) reduced BW in Breed 1 compared to (+WC) ($p \leq 0.05$). Breed 2 responded to MED diet with a 21% BW reduction compared to US ($p \leq 0.05$). Probiotic delivered by either method reduced BW a further 9% ($p \leq 0.05$) without an effect of handling. In both Breed 1 & 2, BW reductions paralleled reductions in voluntary feed intake. Modification of dietary fatty acid composition coupled with satiety inducing probiotic *E. coli* provides a strategy reduce hunger related welfare concerns. Response differences by pullet strain suggests that genetic differences in appetite regulation exist in commercial broiler strains; albeit head-to-head studies are needed to confirm. Strategy effects on egg production and hatchability are needed.

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Key Words: Probiotic; Welfare ; Breeder; Satiety; Well-Being

309P Comparison of four lighting sources on Pekin duck stress, fear, and growth. Abbigail LeBlanc^{1*}, Jessica Rocha¹, Gabrielle House¹, Eric Sobotik¹, Gregory Archer¹, ¹*Texas A&M University, College Station, Texas, United States.*

Ducks and other poultry are known to be sensitive to light spectrum, intensity, flicker, and photoperiod. Limited research has been conducted on how light spectrum affects Pekin duck production and welfare. The objective of this study was to evaluate the effects of four different lighting sources on Pekin duck stress, fear, and growth. Pekin ducks (n=504) were reared in environments illuminated either by a green monochromatic LED (green), a white led (white), a full spectrum LED (FS) or a LED lantern that is typically used in commercial production (lantern) using a 16L:8D photoperiod and intensity corrected for gallilux. Each treatment had 9 replicate pens and all treatments were reared in the same air space. Stress susceptibility was measured using physical asymmetry of three bilateral traits (ASYM; n=240) at 35 days of age. Behavioral fear response was determined using a tonic immobility (TI; n=240) test at 35 days of age. Physical and production parameters were measured using mortality, feed consumption, body weight, pen feed conversion ratio (FCR; n=36). To determine the effects of light spectrum on eye development, left and right eye dimensions (weight, depth, and width; n=80) were measured at 35 days of age. Data were analyzed using GLM, and $P < 0.05$ was considered a significant difference. A LSD post hoc test was used for mean separation. Feed consumption was higher in the white (143 g/day) and green (145 g/day) treatments than both the FS (136 g/day) and lantern (138 g/day) treatments ($P < 0.05$). Day 35 weights were also greater in the white (3.27 kg) and green (3.28 kg) treatments than in both the FS (3.11 kg) and lantern (3.16 kg) treatments ($P > 0.05$). The lantern treatment took less attempts to induce TI (1.4 attempts) than all other treatments (1.8 attempts, $P < 0.05$). While the lantern (242 sec) and FS (229 sec) treatments took longer to right during TI than both the white (157 sec) and green (161 sec) treatments ($P < 0.05$). The lantern treatment had the greatest eye depth (15.1 mm) compared to all other treatments (14.5 mm, $P < 0.05$). No differences were observed in mortality, FCR, asymmetry score, latency to first head movement during TI, time difference from first head movement to righting during TI, eye weight or eye width ($P > 0.05$). The results of this study indicate that duck fear response, growth, feed intake and eye development can be altered by light source and that full spectrum and lantern light may negatively affect these parameters.

Key Words: fear; duck; stress; lighting; welfare

310P A novel light recipe to improve the welfare of broiler chickens. Curtis Leyk^{1*}, Aaron Stephan¹, Jack Tieberg¹, ¹*ONCE Innovations/Signify, Plymouth, Minnesota, United States.*

Lighting represents a promising management tool to improve the well-being of poultry. To date, most studies that compared lighting conditions on broiler welfare outcomes have evaluated individual lighting variables (spectrum, intensity, or schedule), and few studies have compared to conditions used widely in commercial environments. The objective of this study was to find an optimal light recipe for raising broiler chickens, increasing the welfare of the birds without negatively impacting the production parameters. The study was conducted at the ONCE Research Facility and three commercial producers. In each trial a test light recipe was formulated by defining spectrum, schedule, and/or intensity and compared to the standard light recipe used at the facility. In the case of the ONCE Research Facility, 500 Ross 308 or 708 chicks were divided between 10 rooms - 5 rooms utilizing the novel light recipe and 5 with a standard light recipe. Weights and FCR were monitored regularly and welfare measurements were conducted between production days 35 and 55. The welfare measures included: Blood Heterophil:Lymphocyte (H:L) Ratios, Inversion Test, Isolation Test, Emergence Test, and Tonic Immobility (TI). In the commercial trials, broilers were raised according to the integrator's standard protocol, with 2 houses utilizing the novel light recipe and 2 control houses, using flock-source matched chicks. Each house contained between 19,200 and 26,300 birds. Similar welfare tests were conducted, and the integrator's settlement data were used to evaluate production parameters. Student's t-test and two-way ANOVA were used to compare outcomes of each test between light treatment groups. Initial research at the ONCE Research Facility indicated that broiler production was improved when using a specific Red:Green:Blue ratio during the brood phase and a different Red:Green:Blue ratio during the grow phase. This optimized recipe, which will be described, was subsequently evaluated in additional trials at the ONCE Research Facility and at commercial sites, showing improved welfare. An example commercial trial showed improvements in welfare parameters measured: Inversion Test (5.9 vs 6.9 flaps/sec, $p < .001$), Emergence Test (137.7 vs 219.6 s, $p < .001$), Isolation Test (26.6 vs 48.1 vocalizations, $p = .003$), TI (83.3 vs 173.2 s, $p = .001$) and H:L (.748 vs .91, $p = .49$). Results of each trial will be presented, showing a statistically significant improvement ($p < 0.05$) in 6 of 7 Inversion Test, 4/7 Emergence Tests, 7/7 Isolation Test, 6/7 Tonic Immobility, and 1/8 H:L Ratio. The results of this multi-site and multi-flock trial indicate that the welfare of broiler chickens can be improved by utilizing an optimal lighting recipe.

Key Words: Stress; Broiler; Welfare ; Lighting; Behavior Testing

311P Leveraging RFID tracking to monitoring hen movement in cage-free aviaries. Benjamin Lorentz^{1*}, Nabin Neupane¹, Cara Robison², Prafulla Regmi¹, ¹*University of Georgia, Athens, Georgia, United States*, ²*Michigan State University, East Lansing, Michigan, United States*.

The purpose of this study was to investigate space utilization by laying hens in a cage-free aviary housing. Further, the relationship between bird activity pattern and tibia properties and keel fracture incidences were explored. Radio frequency identification (RFID) trackers provide an inexpensive and granular view into behavioral patterns, one drawback, however, they generate massive datasets that cannot be interpreted without processing. For this study, RFID receivers were placed on 3 tiers (bottom, middle, and top) of an aviary hen room that contained nest-boxes on the top-tier. Forty random hens within a section (4 sections per room; 144 hens per section) were outfitted with an RFID chip with a unique identifier from 23 to 46 weeks of age (WOA). At 52 WOA, hens were euthanized, and tibia and keel bones were collected. Keel bones were scored for fractures on a scale of '0' to '2' (0 = no fracture; 1 = single fracture; 2 = multiple fractures) whereas right tibia was imaged using computed tomography (CT) for measurement of total volume and density. Left tibia from the hens were subjected to a 3-point bending test for measurement of maximum force to failure. Time series data for each bird were constructed in R using the XTS package. Transitions from one zone to another were counted over the course of the study and the duration of time a bird spent in each zone before transitioning was measured. Birds were categorized based on the number of transitions they undergo into low (8<=), medium (9-23) and high (>23) activity birds. One-way ANOVA tests were used to determine if there are significant differences between the occurrence of keel-bone fractures between the activity groups. Evidence of keel bone fractures correspond with activity level where low activity birds show evidence of less fractures than high activity birds ($P < 0.05$). Average keel scores were 0.57, 1.14, and 2.0 for low, medium, and high activity birds respectively. Tibia parameters were not affected by the activity levels of the hens. Results indicate the presence of an underlying structure to bird activity. Birds performing high frequency of vertical movement may be spending more time in upper levels with a possibility of falling or over-exerting the keel bone and causing fractures. Further studies, especially linking egg production to activity are warranted.

Key Words: RFID tracking; Hen Welfare; Keel bone fracture; nest-box; bird activity

312P Feedstuff preferences exhibited by American Game cockerels on a restricted diet. Dawshaia Herndon^{1*}, James Wetzel¹, Tatijana Fisher¹, ¹*Lincoln University of Missouri, Jefferson City, Missouri, United States*.

Commercially produced chicken feeds for small flocks are sold in crumble or pellet forms. These diets are designed for

high production commercial chicken strains that benefit from free-choice access to uniformly ground and nutrient dense feedstuffs. However, small flocks often use heritage breed chickens which are less productive and are kept for longer cycles. These flocks may benefit from diets composed of whole, unground feedstuffs which retain their nutrient quality longer and stimulate foraging behavior. However, whole grains may lead to selective consumption by the chicken. The goal of this study was to compare chicken's preferences for coarse particulate feedstuffs (shelled maize corn, black oil sunflower seeds, live mealworms, and a pelleted complete feed) when provided pair-wise choices. Three American Game cockerels were individually housed in rhomboid drop pens (98cm W x 98cm L x 74cm H) on a concrete floor. The cockerels were maintained on a restricted ration (5.5% body weight/day) of Dumor[®] 17% CP All Flock Pellets which was offered at 1000 daily and fully consumed by 1600. After a period of acclimation to the testing regimen, two trials were conducted for each cockerel: one before the daily feeding and the second after all the daily rations had been consumed. During testing, each cockerel was offered 50 particles of two feedstuffs for 30 seconds. Feedstuff options were the pelleted base diet, maize, black oil sunflower seed (BOSS) and live late-instar larval mealworms. All possible combinations were represented for each bird and assigned randomly to enable a Latin Square Combination for all birds and diet combinations. After the test period, the remaining particles were counted and weighed in aggregate. Data were analyzed using paired T-tests. When feedstuffs were offered to cockerels with an empty crop, a preference was shown for maize over pellets. More particles of maize were consumed than pellets ($P < 0.05$), but no significant differences were observed in particles consumed for any other combination of feedstuffs ($P > 0.05$). By weight consumed, more maize was consumed than mealworms or pellets which were consumed more than BOSS ($P < 0.05$). When feedstuffs were offered to cockerels with a full crop, no significant differences were observed in particles consumed for any combination of feedstuffs ($P > 0.05$). By weight consumed, more maize was consumed than the other feedstuffs ($P < 0.05$) which is likely a function of maize particles being heavier. These results indicate that American Game cockerels can exhibit preference for particle types and that preference can be influenced by crop-fill status. However, further study with more complex comparisons and more experimental subjects is needed.

Key Words: heritage breeds; feedstuffs; feed preference; American Game Chicken; cockerels

313P Effects of a proprietary botanical blend feed additive on the welfare of commercial broiler chickens. Hayley Sutherland^{1*}, Yiru Dong¹, Gregory Fraley¹, Marisa Erasmus¹, ¹*Purdue University, West Lafayette, Indiana, United States*.

Broiler chickens are routinely exposed to stressful conditions, such as heat stress, that may decrease their welfare. This preliminary study examined the influence of a proprietary botanical blend feed additive (Probiotech International, Inc.), on welfare measures, walking ability and productivity of broiler chickens exposed to an acute heat stress event during the finishing phase. Broilers (Ross 708; n = 304) were randomly assigned to one of two treatment groups (5 pens/treatment): Phytogenic supplementation: supplemented with the proprietary botanical blend starting at 15 d (PLD); and Control: standard diet with no supplementation (CLD). The feed additive was mixed into feed at an inclusion rate of 250g/tonne and fed until birds were processed at 42 d. Broilers were housed at a density of 27kg/m². Heat stress was applied to both treatment groups from 30 to 32 d, reaching a peak temperature of 34°C. Welfare measures (gait, footpad dermatitis, hock burn, feather cleanliness) and body weight were assessed in 50% of birds at 27 and 39 d; litter quality was also assessed at these timepoints. Feed conversion ratio (FCR) was assessed weekly. Welfare data were analyzed using PROC LOGISTIC; FCR and body weight differences were examined using PROC MIXED (SAS 9.4). At 27 d, all birds received scores of 0 for footpad dermatitis and hock burn and 96% of birds received a score of 0 for feather cleanliness; body weight did not differ among treatment groups. At 39 d, diet significantly influenced hock burn and feather cleanliness and tended to influence footpad dermatitis such that PLD birds had lower odds of having no hock burn than CLD birds (Odds Ratio =0.28, 95% CI (0.12, 0.64), P=0.0023). The odds of having clean feathers were lower for PLD than CLD birds (0.37 (0.18, 0.74), P=0.0049). PLD birds tended to have higher odds of no footpad dermatitis than CLD birds (2.58 (0.86, 7.74) P=0.090). Diet did not influence body weight, FCR, gait or litter score. These preliminary results indicate that providing this botanical blend to broilers housed at 27kg/m² resulted in higher body weights at 39 d, tended to reduce footpad dermatitis compared to control birds housed at the same density, whilst increasing the odds of having hock burn and dirty feathers at 39 d. Further research is needed to examine the influence of the feed additive on broiler behavior as another aspect of broiler welfare.

Key Words: broiler; heat stress; stocking density; welfare; botanical blend

314P The effect of nightlight use on turkey poult welfare and production. Hope Kassube^{1*2}, ¹Penn State, Manheim, Pennsylvania, United States, ²University of Edinburgh, Edinburgh, United Kingdom.

Lighting programs are management tools used to rear poultry. Lights facilitate sight and allow birds to locate feed and water in their environment. Some programs dictate lighting programs for certification. Nightlights may be approved in early poult rearing so poults can find feed during the night. Preliminary data indicated improved poult

performance from d1-7 when nightlights were provided. However, these early improvements were not sustained as body weights of poults reared in pens with no nightlights were similar to those reared with nightlights at d28. There is little data showing if other measures of welfare are affected. The objectives of this study are to determine the effect of a nightlight on turkey poult welfare and production. The hypothesis is that turkeys with a nightlight will have improved growth, but a reduced behavioral repertoire, compared to turkeys without a nightlight. The study was conducted at the Penn State Poultry Education and Research Center (PERC). Turkey hen poults were housed on day of hatch at 35 hens per pen, in 16 pens, spread across four rooms (four pens/room). The rooms were divided into two different light scenarios (with and without nightlight). Both were lit following Global Animal Partnership Standards (GAP) (≥ 50 lux during the day, six hours continuous darkness at night), but the nightlight rooms used a nightlight of three lux during the night. Production data was collected on d28 and d42. On d42, three birds per pen were euthanized and their left tibias were excised for ash determination. Each pen was equipped with one infrared camera that continuously recorded poult behavior. The video recordings were reviewed and scan sampling was performed on all pens, 3 times an hour, one day per week of the study. Behaviors such as walking, feeding, resting, drinking, comfort behaviors, exploratory behaviors, pecking and play were recorded, and a time budget created for each group. An analysis of variance was performed using the GLM procedure in SAS. There was no difference between treatments for feed intake per pen (P=0.452), feed intake per bird (P=0.546), live weight gain per pen (P=0.160), live weight gain per bird (P=0.133), or feed conversion ratio (P=0.112). There was no difference between treatments for mg of ash (P=0.066), ash percent (P=0.211), or mg of ash per kg of body weight (P=0.326). Results indicate that the poults provided with a nightlight did not have increased growth, increased feed intake, an increased feed conversion ratio, or increased bone strength compared to those without a nightlight. Behavioral data are still being analyzed and results will be presented at the conference.

Key Words: behavior; turkeys; welfare; poults; nightlight

315P Virtual interactive online education for poultry industry professionals. Federico Etcheverry^{1*}, Edgar Oviedo-Rondón², ¹*anprocampus.com, Hollywood, Florida, United States*, ²*North Carolina State University, Raleigh, North Carolina, United States*.

Continuing education and extension learning opportunities are essential for professionals working in the diverse sectors of the poultry industry. Although e-learning has been a valid training alternative for more than 20 years, COVID19 pandemic accelerated its adoption. The training offer increased exponentially, and studies indicate e-learning is here to stay. Most of it is based on videos and recorded

PowerPoint presentations, although it is not the best learning resource. Interactive training is the recommended best practice to catch the attention of visual, auditive, and kinesthetic learners, increasing the knowledge retention in the students. This study aimed to determine the value of e-learning as a valid continuing education tool. During 2020 and 2021, the Prestage Dep. of Poultry Science at NC State and anprocampus.com offered three interactive extension courses to the industry: Data Analytics for Poultry and Swine Industries, Feed Formulation and Modeling for Poultry, and Feed Manufacturing. All the theory was presented with interactive self-paced modules that included validation questions, practical exercises, and real production scenarios, with one weekly live online session to answer questions and doubts during the two months of tutoring. Overall, 178 participants from 21 countries attended these extension courses in five editions. One hundred forty-three of these students, who dedicated a minimum of 27 hours each, approved the examination and received their certification. The completion ratio, or the percentage of participants who finished the courses, resulted in 80.33%. This is an extraordinary achievement compared with the 15% of completion of the Massive Online Open Courses or the 60% of completion in corporate training. This completion rate is also remarkable compared with other free certified courses offered at the training platform anprocampus.com which had 41.18% completion. Free courses with paid certification had 33.86% completion and 8.69% of completion with paid certificate. Overall course qualification by the participants was 4.2 out of 5.0, and the average grade obtained in the exams was 7.2 out of 10.00. The answers to the qualitative questions of the surveys support the idea that the students need even more time to keep up the pace of the weekly published content, as the daily responsibilities leave little time for training. This experience shows that high-quality interactive content is well received by professionals and employees working in the industry. Even though the edition of interactive content is quite expensive, it offers a positive extension training experience to the participants while dramatically reducing the costs of traveling and the absence of daily work.

Key Words: feed manufacturing; feed formulation; continuing education; data analytics; e-learning

316P Debugging poultry sustainability: Advancing insects as an alternative protein source through a USDA Grand Challenge Research Approach. Kelsy Robinson^{1*}, Komala Arsi², Kristin Duffield³, Tawni Crippen⁵, Kim Cook⁴, Annie Donoghue², ¹USDA - ARS, Mississippi State, Mississippi, United States, ²USDA-ARS, Fayetteville, Arkansas, United States, ³USDA-ARS, Peoria, Illinois, United States, ⁴USDA-ARS, Beltsville, Maryland, United States, ⁵USDA-ARS, College Station, Texas, United States.

Poultry is one of the most widely consumed protein products worldwide due to its low price and consistent quality. Demand for poultry products will continue to

increase as the global population rises. However, meeting this demand is complicated by predicted negative impacts of climate change on animal production, including decreased feed supply and increased disease incidence. Innovative solutions are needed to sustain poultry production and ensure a safe, reliable protein supply for future generations. Since insects are a normal protein source in the wild avian diet, industrialized production of insects and insect meal has the potential to be such a solution by substituting for plant-based protein currently used for commercial poultry diets. Insect meals are rich in high quality protein, unsaturated fats, vitamins and minerals. They can be raised sustainably in a variety of climates due to their low water requirements, high variability in rearing substrates and feed efficiency, low greenhouse gas emissions, and high reproductive potential. Additionally, when insect meal is used as an alternative protein and/or fat source in poultry feed it does not negatively affect poultry health, growth, or carcass characteristics; and it possess antimicrobial compounds such as chitin, lauric acid, and antimicrobial peptides. Even with these positive indicators, additional work is needed to ensure insect meal is adopted as a viable product by the poultry industry. The USDA ARS has implemented a novel approach to coordinate researchers to synergistically forward the development of insect meal as a novel protein source for poultry diets. The Insect Grand Challenge initiated in 2021 includes 42 ARS scientists representing 11 National Programs and 22 Research Units across the US and Greece, as well as academic partners and industry stakeholders. This team is working to develop a model of insect production that incorporates economics, sustainable ecological insect production systems, infrastructure and food animals (poultry, aquaculture and livestock) to investigate the value-add and quality assurance of rearing insects on waste diets. Research includes innovation of insect production facilities and processing techniques, refining of insect nutrition and production, development of genetically-improved insect livestock in an effort to assist industry with upscaling production to meet the demands of poultry diets. The effect of insect meal diets on poultry production, disease resistance, and food safety is being thoroughly investigated along with optimization of inclusion levels. Data gained from this project will provide valuable information to support the U.S. insect ag industry as a new livestock commodity.

Key Words: Sustainability; Insects; Insect Meal; Alternative Protein

317P Update of the Virginia Tech Research Education Programs: A Scientific Approach to Research Training for URM's. Edward Smith^{1*}, Luke Achenie¹, Zac Mackey¹, ¹Virginia Tech, Blacksburg, Virginia, United States.

A recent biannual report by the National Science Foundation (<https://nces.nsf.gov/pubs/nsf21321/>) showed that only 9% of academic jobs are held by underrepresented minorities, even though they are 33% of the US population.

Additionally, our land grant system of graduate training has always been faculty-driven and one recruit at a time. This approach is similar to the one gene:one phenotype traditional approach that has now been replaced by a system-approach to identifying the genes that affect economic traits. The traditional approach will not help reduce the paucity of URM PhD trainees in the biomedical and agricultural sciences and STEM research careers. In 2003 and 2007, through competitive funding from the National Institutes of Health, Virginia Tech established the Post-baccalaureate Research Education Program (PREP) and the Initiative to Maximize Student Development (IMSD), respectively. Our training programs are based on the hypothesis that a cohort approach to graduate education have better outcomes than the professor-driven tradition of the land grant system. A total of 219 trainees have participated in these research education programs. The undergrad institutions of origin have been national and The US Territories and Puerto Rico. The trainees have pursued research training in diverse disciplines including physiology, biomedical engineering, neuroscience, microbiology, and genomics/genetics. The training plan has included innovative approaches such as an Ombudsperson for graduate education, retrospectives by scientists as “scientific journeys” on YouTube, peer and near-peer mentoring, and a committed engagement with alumni for their lived experiences and to continue impacting their careers. Additionally required courses include “Effective Grant Writing” and Scientific Writing. The courses help us to not only provide additional training in “soft skills,” but to stay in regular contact with trainees in the early months of the PhD. Together, these efforts have resulted in 88 PhDs, 3 MDs, 30 MS and 1 DPharm. Trainees are pursuing diverse careers including 13 in tenure-track academic positions, one of whom was recently tenured. Our recent rate continues to be above 75%. Beyond research skills, our trainees acquire networking, mentoring, and communication (written and oral) skills that have contributed to their successes. Our creativity as scientists, I will argue, has made these successes more likely in a department not generally considered biomedical or behavioral. Our future efforts with these research training programs will include the use of “lived experiences” by our distinguished alumni as a training tool.

Key Words: graduate education; Research education; cohort approach

318P An update on transcriptomes of an array of chicken ovary, intestinal, and immune cells and tissues. Theros Ng^{1*}, R Hawkins², Yvonne Drechsler¹, ¹*Western University of Health Sciences, Pomona, California, United States*, ²*University of Washington, Seattle, Washington, United States*.

While the chicken (*Gallus gallus*) is the most consumed agricultural animal worldwide, the chicken transcriptome remains understudied. We have characterized the

transcriptome of 10 cell and tissue types from the chicken using RNA-seq, spanning intestinal tissues (ileum, jejunum, proximal cecum), immune cells (B cells, bursa, macrophages, monocytes, spleen T cells, thymus), and reproductive tissue (ovary). Previously, using the STAR aligner with GRCg6a, Ensembl annotation release 98, genome-build-accession NCBI:GCA_000002315.5, we detected 17,872 genes and 24,812 transcripts across all cell and tissue types, and representing 73% and 63% of the current gene annotation, respectively. Because of the newly released chicken genome and annotations: GCF_016699485.2_bGalGal1.mat.broiler.GRCg7b, GCF_016700215.2_bGalGal1.pat.whiteleghornlayer.GCg7w, we have realigned the raw reads from our data. The objective of this work is to demonstrate aligner differences so that future studies on the chicken transcriptome can be aware of the limitation of some aligners. The layer and broiler genome references were obtained using phased assembly from the F1 female progeny of the parental cross of a female layer and a male broiler. Overall, HISAT2 aligned fewer genes by 40-44% and transcripts by 35-44% compared to STAR. This is significant because as the total number of genes and transcripts will increase over the new releases, and more of the genes and transcripts will be missed by HISAT2. Additionally, the inaccurate mapping by HISAT2 compared to STAR may result in mismatching or missing smaller transcripts such as miRNA. Since the goal of this project in the FAANG initiative is to improve the annotation of the chicken, a more accurate aligner such as STAR is necessary. However, HISAT2 processing speed was significantly higher by 73% (STAR: 59 minutes vs HISAT2: 16 minutes for 50 million reads paired-ended fastq alignment) and the minimum memory requirement was lower by 50% (STAR: 128 Gb vs HISAT2: 64 Gb). We detected 13,288 genes and 23,274 transcripts using the GRCg7b and 13,305 genes and 24,054 transcripts using the GRCg7w NCBI genome and annotation releases. The number of genes and transcripts detected by HISAT2 was similar between all the chicken genome releases (GRCg6a, GRCg7b, and GRCg7w) with fewer genes and transcripts detected in the newer releases (GRCg7b and GRCg7w). In conclusion, although HISAT2 performed faster than STAR, STAR performs better in terms of accurately mapping the chicken genome.

Key Words: RNA-seq; Reproduction; Transcriptome; Intestine; Immune

319P Highly pathogenic avian influenza virus H5N1 stimulate the MAPK signaling pathway in the chicken trachea. Yeong Ho Hong^{1*}, Thi Hao Vu¹, Yeojin Hong¹, Anh Duc Truong², Sooyeon Lee¹, Jubi Heo¹, Hyun Lillehoj³, ¹*Chung-Ang University, Anseong, Gyeonggi-do, Korea (the Republic of)*, ²*National Institute of Veterinary Research, Hanoi, Viet Nam*, ³*USDA- ARS, Beltsville, Maryland, United States*.

The highly pathogenic avian influenza virus (HPAIV) is a threat to the poultry industry and economy and remains a potential source of pandemic infection in humans. Antiviral genes are considered a crucial factor for HPAIV resistance. Therefore, in this study, we investigated gene expression related to the MAPK signaling pathway by comparing non-infected, HPAI-infected resistant, and Ri chicken lines of trachea tissue. Ri chickens of resistant (*Mx/A; BF2/B21*) and susceptible (*Mx/G; BF2/B13*) lines were selected by genotyping the *Mx* and *BF2* genes. These chickens were then infected with HPAIV H5N1, and their trachea tissues were collected for RNA sequencing and were confirmed by quantitative real-time polymerase chain reaction. The differentially expressed genes (DEGs) between the two selected biological conditions were analyzed using the Cuffdiff software. Assignment to significance was determined using a threshold ($|\text{Fold-Change}| \geq 2$ and $p\text{-value} \leq 0.05$). A total 1,794 DEGs were observed between control and H5N1-infected resistant chickens, 432 DEGs between control and infected susceptible chickens, and 1,202 DEGs between infected susceptible and infected resistant chickens. Most of the DEGs in the HPAI-resistant and HPAI-susceptible comparisons were related to the MAPK signaling pathway. The expression levels of MAPK signaling pathway-related genes (*MyD88*, *NF-κB*, *AP-1*, *c-fos*, *Jun*, *JunD*, *MAX*, *c-Myc*), cytokines (*IL-1β*, *IL-6*, *IL-8*), type I interferons (*IFN-α*, *IFN-β*), and IFN-stimulated genes (*Mx1*, *CCL19*, *OASL*, and *PRK*) were higher in H5N1-infected than in non-infected resistant chickens. *MyD88*, *Jun*, *JunD*, *MAX*, cytokines, chemokines, IFNs, and IFN-stimulated expressed genes were higher in resistant-infected than in susceptible-infected chickens. In conclusion, resistant Ri chickens showed higher antiviral activity compared to susceptible Ri chickens, and H5N1-infected resistant Ri chickens had immune responses and antiviral activity by upregulating cytokines, chemokines, interferons, and IFN-stimulated genes, which may have been induced through the MAPK signaling pathway in response to H5N1 infection.

Key Words: RNA sequencing; H5N1; HPAIV; MAPK signaling pathway; Ri Chicken

320P Effect of tannin-free grain sorghum on intestinal histomorphometry and gene expression of jejunal mucosa of broiler chickens under necrotic enteritis challenge. Alissa Moritz¹, Stevin Wilson¹, Santiago Sasia^{1*}, Mireille Arguelles-Ramos¹, ¹*Clemson University, Clemson, South Carolina, United States.*

The objective of this study was to explore the potential of tannin-free grain sorghum varieties as nutraceuticals. A total of 512 Cobb male chicks were placed in battery cages and fed 4 dietary treatments (corn, red/bronze, white/tan, or U.S. No. 2 sorghum) and grouped by challenge method (challenged with EM/CP or unchallenged) until 28-d-old. On day 14, challenged birds were orally inoculated with ~5,000 oocysts of EM, and on days 19, 20, and 21, birds

were given a broth culture of CP with ~108 CFU/ml once daily. On day 28, 1 bird from 4 of the 8 cages/treatment was randomly selected, weighed, and euthanized to collect intestinal tissue for histomorphometric analysis and jejunal mucosa for gene expression analysis. Data were analyzed as a 2 x 4 factorial design with factors of challenge and grain types defining the treatments and their interaction. Each cage was used as the experimental unit for analysis. Two-way ANOVA followed by Fisher's LSD procedure was used to determine if differences existed among the treatment means. Statistical significance was based on a $P\text{-value} < 0.05$. Gene expression was assessed by RNA-sequencing analysis on jejunum mucosa to determine changes in the transcriptome. Total RNA was extracted using a standard TRIzol method. After library preparation, these were sequenced on an Illumina NovaSeq 6000. Genes were considered differentially expressed (DEG) with a false discovery rate (FDR) < 0.05 and $|\log_2\text{Fold Change (FC)}| > 1$. ClusterProfiler was used to perform gene set enrichment analysis (GSEA) using gene ontology (GO). Finally, a targeted analysis using LC-MS/MS of 24 select metabolites determined the relative quantification of each compound to an internal standard (13C6 resveratrol). There were no significant differences among treatments on villi height, villi width, crypt depth, and crypt:villi ratio. mRNA-sequencing revealed 152 DEGs in challenged red/bronze when compared to challenged corn. GSEA using GO showed an upregulation in defense response to biotic stress in challenged red/bronze when compared to challenged corn birds, with *LYG2*, *AVD*, and *NOS2* genes among those upregulated. The Reactome pathway associated with metal sequestration by antimicrobial proteins was over-represented among genes upregulated under the red/bronze treatment. The greatest relative quantities of flavonoid and phenolic compounds in red/bronze and U.S. No. 2 grain sorghum were hydroxycoumarin, caffeic acid, apigeninidin, formononetin, and luteolinidin compared to the white/tan variety. Therefore, gene expression and relative quantity of select polyphenolic compounds can provide insight into the beneficial or detrimental effects on intestinal health when broilers are fed tannin-free grain sorghum.

Key Words: broilers; gene expression; necrotic enteritis; intestinal health; grain sorghum

321P Effects of microinjection of growth factors on Rathke's pouch development in chicken embryos. Scott Klug^{1*}, Tom Porter¹, ¹*University of Maryland, College Park, Silver Spring, Maryland, United States.*

During embryogenesis, oral ectoderm (OE) invaginates cranially to form Rathke's pouch (RP), the precursor structure to the anterior pituitary gland, which secretes hormones regulating growth, metabolism, reproduction, and stress responses. Physical contact between OE and neural ectoderm (NE) and signaling between these two tissue layers is vital to proper pituitary development. However, a complete model explaining the mechanisms regulating

pituitary development and RP formation is lacking. Retinoic acid (RA) has been shown to regulate pituitary-specific transcription factor Prop1, which is essential for correct pituitary morphology and the production of hormones. Sonic Hedgehog (SHH) has been shown to be essential for pituitary proliferation and cell differentiation. However, most previous research in this area has been conducted in laboratory mice. Our current research is aimed at evaluating direct effects of RA and SHH on RP morphology in the chicken embryo. RP develops by 60 hours (h) of incubation in the chicken. Phosphate-buffered saline containing green food coloring (PBS-G) and PBS-G with Smoothed agonist (SHH agonist; SAG), Jervine (SHH antagonist), RA, and retinoic acid receptor antagonist (RARA) were microinjected into developing chicken embryo forebrains *in ovo* after 36 h of incubation (10^{-7} M solutions; 7-8 embryos/treatment). Injection sites were visualized using a dissecting microscope. The embryos were then returned to the incubator and collected 24 hours later. Approximately 85% of the embryos survived the procedure, and only embryos with 10-11 somites present at the time of injection were processed further (3 embryos for each treatment). Embryos were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Mid-sagittal sections were identified, and RP length, tissue area, and total area (including the space within the pouch) were measured in the mid-sagittal sections using ImageJ software in a researcher-blinded approach to prevent bias. Unblinded data were then analyzed in SAS using PROC ANOVA, and statistical significance was determined by Tukey's HSD. Compared to controls (PBS-G), SAG treatment reduced RP length, tissue area, and total area by 47%, 51%, and 65%, respectively ($P < 0.05$; $n=3$). Jervine-treated embryos were not statistically different from controls. RA- and RARA-treated embryos showed reduced RP length, tissue area, and total area compared to control. However, effects of both treatments were not statistically significant. We are currently evaluating other potential signaling molecules and pathways, in order to better understand the signaling mechanisms between NE and OE necessary for pituitary development in the chicken.

Key Words: embryo; pituitary; Rathke's pouch; retinoic acid; sonic hedgehog

322P Effect of cyclic heat stress on hypothalamic oxygen homeostasis and inflammatory state in the Red Jungle Fowl and three modern broiler lines. Alison Ramser^{1*}, Giorgio Brugaletta², Elizabeth Greene¹, Craig Maynard¹, Travis Tabler¹, Federico Sirri², Nicholas Anthony¹, Sara Orlowski¹, Sami Dridi¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Alma Mater Studiorum – University of Bologna, Bologna, Italy.

Heat stress is an ever-increasing threat to the performance and welfare of the modern broilers. In addition to decreased weight gain and increased water consumption, heat stress significantly decreases feed intake. This depression is one

of the leading factors in the loss of performance seen under heat stress conditions and has been shown to be partially centrally regulated. In the pursuit of better understanding the role the central nervous system plays in heat stress induced feed intake depression, we compared the hypothalamic expression of a newly involved pathway, hypoxia/oxygen homeostasis, in heat-stressed modern broilers and jungle fowl, given the differences in heat stress tolerance across these populations. The present study used three populations of broilers (slow growing ACRB from the fifty, moderate growing 95RB from the ninety, and modern fast growing MRB from 2015) and unselected Jungle fowl birds. All birds were exposed to cyclic heat stress (36°C, 9h/day for 4 weeks) in a 2x4 factorial experimental design before protein and mRNA was isolated from the hypothalamus under all treatments. The expression of target genes and proteins was determined by real-time quantitative PCR and Western blot, respectively, with two-way ANOVA analysis on GraphPad software. Heat stress showed to increase the expression of certain hemoglobin subunits, oxygen homeostasis signaling cascades, and heat shock proteins in a line-dependent manner ($P < 0.05$). Additionally, there was a significant effect of line on HIF-1 α ($P = 0.02$) with higher expression in JF compared to other modern broilers especially under HS conditions. As a whole, this study is the first to implicate the hypothalamic expression of hypoxia- and oxygen homeostasis-associated genes as well as their up- and down-stream mediators in chickens with more pronounced differences in susceptible modern lines compared to their ancestor jungle fowl. These results point to the interconnection of hypoxia, thermotolerance, and feed intake and provides a basis of future research into this relationship.

Key Words: broilers; heat stress; hypothalamus; jungle fowl; oxygen homeostasis

323P Gene expression of umami taste compounds receptors in the gastrointestinal tract of broiler chickens. Francisca Díaz-Avilés¹, Paloma Cordero¹, Paulina Torres¹, Miguel Guzmán², Sergio Guzmán-Pino^{1*}, ¹Universidad de Chile, Santiago, Metropolitana, Chile, ²Universidad de Las Américas, Santiago, Chile.

Broiler chickens seem to have developed an acute sense of taste which enable them to distinguish at least five of the six primary tastes. Among them, umami taste compounds are linked to the protein fraction of diets that are perceived through a different receptor in oral and extra-oral tissues. The aim of this study was to evaluate the relative gene abundance associated with umami taste compounds receptors in the gastrointestinal tract (GIT) of broilers. A total of sixteen broiler chickens (Ross 308) were randomly selected at 7 and 26 days of age for gene quantification in ten different sections of the GIT (tongue base, palate base, crop, proventriculus, gizzard, duodenum, jejunum, ileum, cecum, and colon). The relative gene abundance of T1R1, T1R3, mGluR1, mGluR4, GPR92, GPR139, GPRC6A, and

CaSR was analyzed by real-time PCR. Samples RNA extraction and subsequent procedures followed six steps according to commercial manufacturer instructions. Data were analyzed with ANOVA by using RStudio considering the effects of chickens' age, tissue, and age x tissue interaction (AxT). The expression of T1R1 was significantly higher ($p < 0.001$) in 7 vs 26-day-old chickens. A tissue effect was also observed for this gene ($p < 0.001$), while no differences ($p > 0.05$) were found at AxT. The expression of T1R3 was also significant between ages ($p < 0.001$), tissues ($p < 0.001$), and AxT ($p < 0.001$). The mGluR1 and mGluR4 genes showed no significantly different expression in 7 vs 26-day-old groups ($p = 0.37$ and $p = 0.24$, respectively). A tissue effect was detected for mGluR4 expression ($p < 0.001$). Both mGluR1 and mGluR4 showed a significant AxT effect ($p < 0.05$). The GPR92 gene showed significant expression between ages ($p < 0.001$), tissues ($p < 0.001$), and AxT ($p < 0.001$). The CaSR gene showed significant age ($p < 0.05$) and AxT effects ($p < 0.001$). The GPRC6A and GPR139 gene were not found to be expressed in broilers' GIT. It is concluded that the expression of umami taste compounds receptors varies with chickens' age and GIT section. Most of the receptors here analyzed showed higher expression at the initial stage of broilers (7-day-old), which suggests a higher umami acuity in young birds.

Key Words: broiler chicken; gastrointestinal tract; gene expression; taste receptors; umami taste

324P Expression profile of water channel- And noncoding RNA biogenesis-related genes in heat-stressed modern broilers and their ancestor Red Jungle Fowl. Wafa Youssfi¹*, Elizabeth Greene¹, Travis Tabler¹, Sara Orłowski¹, Nicholas Anthony¹, Sami Dridi¹, ¹University of Arkansas, Fayetteville, Arkansas, United States.

Genetic selection for high growth rate has resulted in spectacular progress in feed efficiency. As feed intake and water consumption are associated and both are affected by environmental conditions, we undertook the present study to evaluate water consumption and its underlying molecular mechanisms in three broiler-based research lines and their ancestor jungle fowl (JF) under thermoneutral (TN) and heat stress (HS) conditions. Slow growing ACRB from the 1950s, moderate growing 95RB from the 1990s, and modern fast growing MRB from 2015 and unselected chickens (JF). were exposed to daily chronic cyclic HS (36°C, 9h from 29 to 56 days) or TN (24°C) in a 2x4 factorial experimental design (150 birds/group). Hypothalamus of 6 birds randomly selected from each combination of environmental temperature and line were collected for analysis. The expression of target hypothalamic genes was determined by real-time quantitative PCR and analyzed by two-way ANOVA, and results were considered statistically significant at $P < 0.05$. HS reduced body weight and increased WC in 95RB and MRB but not in JF or ACRB. In the hypothalamus, chronic

HS up-regulated the expression of AQP (2, 9, 10, and 11) in all lines. AQP1 was down-regulated only in JF and AQP3 decreased in MRB and increased in both ACRB and 95RB. In contrast to JF, AQP4 was up-regulated in ACRB ($p = 0.01$). A significant interaction between line and temperature has been noticed for the AVP ($p = 0.04$). HS did not affect AVP in JF, ACRB and 95RB but significantly decreased it in MRB ($p = 0.03$). AVPR2 was up-regulated in JF and decreased in the modern chickens. AVPR1B was heightened in four populations. HS reduced AVPR1A in MRB and elevated it in 95RB. AT1 and AT2 were down-regulated in JF. The expression of AGE, AGT and renin was increased in MRB. Also, the expression of RNase family enzymes was altered by chronic HS in a line-dependent manner with DICER1 being up-regulated and Drosha down-regulated in all the populations except for 95RB. DGCR8 was increased in JF, ACRB and 95RB. There is no difference in the expression of AGO2 except for an up-regulation in JF under HS. This is the first report showing the effect of HS on water channel- and noncoding RNA biogenesis-related hypothalamic genes in modern chicken population and their ancestor JF.. These results provide a basis of future research to identify new molecular mechanisms involved in adaptation of WC under HS.

Key Words: broilers; gene expression; heat stress; water channels; miRNA biogenesis

325P Effect of herbal adaptogen on feeding-related hypothalamic neuropeptides in heat-stressed broilers. Maryam Afkhami Ardakani¹*, Elizabeth Greene¹, Sami Dridi¹, ¹University of Arkansas, Fayetteville, Arkansas, United States.

Heat stress is a major issue in the poultry industry, having numerous negative consequences for chicken productivity. One of these is a decrease in feed intake, which results in higher mortality and poor growth performance. Herbal adaptogens, plant extracts that are also known as stress response modifiers, are metabolic regulators that improve an organism's ability to adapt to environmental stresses and minimize damage from such stressors. Previously, we published data indicating that herbal adaptogen supplementation increased feed intake under heat stress conditions. Therefore, we hypothesized that dietary herbal adaptogen supplementation may exert these effects through alteration of the expression of hypothalamic neuropeptides involved in appetite and feed intake in heat-stressed broilers. A total of 720 male broiler cobb 500 chicks were randomly assigned to 12 chambers for three different diet treatments. In treatment 1, broilers were fed with a corn-soybean-based diet as a control group. The broilers in treatments 2 and 3 were supplemented with the herbal adaptogen in two different doses in control diets 500 g/1000 kg (NR-PHY-500) and 1 kg/1000 kg (NR-PHY-1000), respectively. Broilers in 9 chambers were exposed to 35 °C for 8 hours (cyclic heat stress condition) from d29 to d42, while 3 chambers were maintained at 24 °C (thermoneutral

condition) for all 42 days. The real-time qPCR was used to evaluate the gene expression of 22 hypothalamic neuropeptides that play a vital role in feed intake and appetite, and the data were analyzed using ANOVA. Gene expression of melanocortin receptor 4 increased significantly in the adaptogen groups compared to the control ($P = 0.0164$). Furthermore, while the expression of orexin showed a clear tendency toward significance ($P = 0.0594$), the majority of neuropeptides' expressions remained unchanged. In conclusion, these findings suggested that adding adaptogen supplementation to broilers' diet could be a promising way to reduce the negative effects of heat stress by modulating hypothalamic genes that control appetite and feed intake.

Key Words: broilers; feed intake; heat stress; herbal adaptogen; hypothalamic neuropeptides

326P Gene expression of fatty and sweet taste compounds receptors in the gastrointestinal tract of broiler chickens. Paloma Cordero¹, Francisca Díaz-Avilés¹, Paulina Torres¹, Miguel Guzmán², Sergio Guzmán-Pino^{1*}, ¹Universidad de Chile, Santiago, Metropolitana, Chile, ²Universidad de las Americas, Santiago, Chile.

It has been described that birds have the ability to select nutrients through receptors expressed in taste cells and extra-gustatory tissues. Between the taste modalities they can detect, fatty taste compounds are linked to lipids and sweet taste compounds to carbohydrates present in the diets. The aim of this study was to evaluate the relative gene abundance associated with fatty and sweet taste compounds receptors in the gastrointestinal tract (GIT) of broilers. A total of sixteen broiler chickens (Ross 308) were randomly selected at 7 and 26 days of age for gene quantification in ten portions of the GIT (tongue base, palate base, crop, proventriculus, gizzard, duodenum, jejunum, ileum, cecum, and colon). The relative gene abundance of GPR41, GPR43, GPR120 (fatty), SGLT-1 and T1R3 (sweet), was analyzed by real-time PCR. Samples RNA extraction and subsequent procedures followed six steps according to commercial manufacturer instructions. Data was analyzed with ANOVA by using RStudio considering the effects of chickens' age, tissue and age x tissue interaction (AxT). The expression of GPR41 showed no significant differences between tissues ($p = 0.091$), ages ($p = 0.612$) and AxT ($p = 0.104$). In addition, a significant difference was observed in the expression of GPR43 between different tissues ($p < 0.001$) and AxT ($p < 0.001$), but not between ages ($p = 0.055$). Similarly, GPR120 showed significant differences in expression between different tissues ($p < 0.001$) and AxT ($p < 0.001$), but not between ages ($p = 0.571$). Significant differences were also observed in the expression of SGLT-1 between different tissues ($p < 0.001$), 7 vs 26-day-old groups ($p < 0.001$) and AxT ($p < 0.001$). Finally, significant differences were found in the expression of T1R3 between different tissues ($p < 0.001$), 7 vs 26-day-old groups ($p = 0.001$) and AxT ($p = 0.005$). It is concluded that the

expression of fatty and sweet taste compounds receptors varies with chickens' age and GIT section. The higher expression at the initial stage (7-day-old) showed by some genes could be associated with an optimizing mechanism for sugary nutrients intake in diets.

Key Words: broiler chicken; gastrointestinal tract; gene expression; taste receptors; fatty and sweet taste

327P Effects of L-tryptophan and 1,25-dihydroxycholecalciferol on the osteogenic differentiation of mesenchymal stem cells isolated from compact bones of broilers and layers. Qianru Hui^{1*}, Xiaoya Zhao¹, Martin Nyachoti¹, Karmin O^{1,2} Chengbo Yang¹, ¹University of Manitoba, Winnipeg, Manitoba, Canada, ²The Canadian Centre for Agri-Food Research in Health and Medicine, Boniface Hospital Research Centre, Winnipeg, Manitoba, Canada.

Poultry birds are vulnerable to bone problems throughout their life or production period due to a rapid growth rate in broilers and an active laying cycle in layers. The calcium-sensing receptor (CaSR) and vitamin D receptor (VDR) are important in calcium and bone metabolism. The objective of this study was to investigate the effect of ligands of CaSR (L-Trp) and VDR [1,25-dihydroxycholecalciferol, 1,25(OH)₂D₃] on the proliferation and osteogenic differentiation of mesenchymal stem cells (MSCs) isolated from compact bones in 14-day-old Ross 308 chickens and Dekalb pullets. Results showed that the high doses (10 and 15 mM) of L-Trp inhibited proliferation but promoted osteogenic differentiation, and 5 mM L-Trp had a balanced effect between proliferation and osteogenic differentiation in broiler and layer MSCs. Therefore, 5 mM L-Trp was selected for further experiments. The broiler and layer MSCs were then treated with (1) osteogenic medium, osteogenic medium supplemented with (2) 1 nM 1,25(OH)₂D₃, (3) 2.5 mM Ca²⁺, (4) 2.5 mM Ca²⁺ + 5 mM L-Trp, (5) 2.5 mM Ca²⁺ + 5 mM L-Trp + 1 μM NPS-2143, respectively for 7 days. All data were expressed as mean ± SEM with at least three biological repeats. Statistical significance was determined using one-way ANOVA followed by Dunnett's multiple-comparison test. P -value < 0.05 was considered statistically significant. The 5 mM L-Trp significantly inhibited the proliferation of broiler and layer MSCs on day 7 ($P < 0.05$), but 1 nM 1,25(OH)₂D₃ significantly promoted the proliferation of layer MSCs ($P < 0.05$). The osteogenic differentiation process in layer MSCs was faster than that in broiler MSCs. Only 2.5 mM Ca²⁺ + 5 mM L-Trp group significantly increased the mineralization process during osteogenic differentiation in broiler MSCs ($P < 0.05$). The NPS-2143 supplementation did not block the positive effect of 5 mM L-Trp on ALP activity and the mRNA expression of osteogenic genes. In layer MSCs, Ca²⁺ alone significantly facilitated mineralization and ALP activity after 7-day osteogenic differentiation ($P < 0.05$). However, different treatments selectively regulated the mRNA expression

of *CaSR*, *VDR*, osteogenesis-related genes, β -catenin, and *NFATc1* without a consistent trend. Taken together, *CaSR* and *VDR* were expressed in boiler and layer MSCs, and L-Trp and 1,25(OH)₂D₃ can regulate proliferation and osteogenic differentiation in both broiler and layer MSCs depending on the modulator doses and treatment time, which provided evidence that *CaSR* and *VDR* could be potential targets to regulate bone formation and remodeling in broilers and layers.

Key Words: L-tryptophan; Calcium-sensing receptor; vitamin D receptor; osteogenic differentiation; chicken mesenchymal stem cells

328P Hypoxia decreases proliferation and differentiation of chick satellite cells. Usuk Jung^{1*}, Minjeong Kim¹, Ahmed Bettaieb¹, Brynn Voy¹, ¹*University of Tennessee, Knoxville, Tennessee, United States.*

Localized tissue hypoxia is linked to the development of breast muscle myopathies in broilers. Lesions in myopathies are characterized by fibrotic, fatty loci that develop within the pectoralis major (p. major). Development and maintenance of healthy muscle fibers rely on the myogenic potential of satellite cells (SCs), muscle stem cells that differentiate to form myotubes. The objective of this study is to determine if hypoxia influences the proliferation, myogenic potential, and metabolism of SCs in ways that could contribute to formation of myopathic lesions. Primary SCs were isolated and purified from p.major of 5-day-old male chicks (Cobb 700) and exposed to hypoxia (1% O₂) in vitro to evaluate the effects on proliferation, myogenic potential, cellular metabolism, and lipid accretion. XTT and CCK-8 assays ($n = 8$) were performed to measure the proliferation rate. Myogenic potential was evaluated by staining fixed myotubes ($n = 3$) with HEMA-3 and using image analysis Image J to calculate the fusion index (%) and the number of nuclei per myotube. Myotubes were also stained with myosin to visually evaluate differentiation. Western blotting for key proteins related to myogenic differentiation, energy homeostasis, fatty acid synthesis, and apoptosis was used to determine if hypoxia influences substrate metabolism (Pooling 3 biological replicates). Lipid accumulation was determined by immunofluorescent staining of myotubes ($n = 3$) with BODIPY (493/530), to visualize lipid droplets, and DAPI to visualize nuclei. Means are compared by one-way ANOVA with posthoc Tukey's HSD test. Differentiating SCs were exposed to hypoxia for 24 or 48 h or maintained under standard culture conditions. The effects of hypoxia were confirmed based on stabilization of HIF1 α protein. Hypoxia exposure for 48 h suppressed the proliferation of SCs ($P < 0.05$). Hypoxia significantly influenced the myogenic differentiation of satellite cells, with decreased fusion index and suppressed myotube formation compared to normoxia. Effects of hypoxia (24 h) on both cell proliferation and myogenic potential were rescued by 24 h normoxia recovery ($P < 0.05$). Western blotting showed that hypoxia diminished

myogenin expression, activated AMPK, upregulated lipogenic proteins, and increased molecular markers of apoptosis. Visual analysis also suggested that hypoxia promoted the accumulation of lipid droplets in myotubes, although there was no significant difference in intensity of BODIPY staining. In conclusion, these data further support the potential for inhibition of myogenesis and accumulation of lipids in breast muscle under hypoxia. Additional measures to identify the mechanisms of myogenesis and adipogenesis by hypoxia are underway.

Key Words: Broiler; Wooden Breast; Hypoxia; Myogenesis; Satellite Cells

329P Evaluation of different DNA extraction methods for the detection of *Clostridium perfringens* by loop-mediated isothermal amplification (LAMP) assay. Deepa Chaudhary^{1*}, Linan Jia¹, Anuraj Sukumaran¹, Wen-Hsing Cheng¹, Aaron Kiess², Chuan-Yu Hsu¹, Li Zhang¹, ¹*Mississippi State University, Mississippi State, Mississippi, United States,* ²*North Carolina State University, Raleigh, North Carolina, United States.*

Clostridium perfringens (*C. perfringens*) is a gram-positive bacterium that causes necrotic enteritis, leading to substantial economic losses in the poultry industry. The development of new diagnostic assays and in-field diagnostic tools are essential to prevent the spread of *C. perfringens*. The loop-mediated isothermal amplification (LAMP) assay is a promising new in-field testing tool to detect *C. perfringens*. There is, however, the need to standardize the assays with a simple, inexpensive, and equipment-minimizing of DNA extraction method for increased efficiency and adaptability to low-resourced laboratories. Thus, the objective of this study was to select a convenient DNA extraction method for the LAMP assay to detect *C. perfringens*. Four DNA extraction methods: Spin-column (SC), Magnetic Beads (MB), Dipstick (DS), and Hotshot (HS) were evaluated by using the genomic DNA extracted from four *C. perfringens* strains based on the DNA quality control standards (yield, purity, and quality) and the performance of two DNA downstream applications, LAMP and PCR assays. Additionally, the sensitivity of the LAMP assay was verified with DNA extracted from broiler feces artificially spiked with 10⁷ CFU/g *C. perfringens* using optimized SC, DS, and HS extraction protocols. The SC and MB extracted DNA had higher yields with better purity and quality. The genomic DNA extracted from the SC method exhibited the best performance with successful amplification in both LAMP and PCR from all tested strains, whereas MB-extracted DNA succeeded in both LAMP and PCR from 3 out of 4 tested strains. DS or HS-extracted DNA failed to amplify in LAMP and PCR from all strains, but the amplification failure was partially resolved using diluted DNA in the assays, suggesting impurities' interference in undiluted DNA samples on LAMP and PCR amplification. Briefly, diluted DS-extracted DNA allowed successful LAMP

amplification from 2 tested strains, but not PCR amplification, while the HS method provided successful amplification in LAMP from all tested strains and in PCR from 3 tested strains. In the sensitivity assessment of the LAMP assay, the SC-extracted DNA had the highest sensitivity, whereas DNA from DS and HS methods had lower sensitivity for the assay. Conclusively, the SC extraction method yields DNA of the highest purity and quality and has the best LAMP and PCR performance. However, regarding labor, time, cost, and required equipment, the HS method is the most convenient method to obtain DNA with the diagnostic assay capability in limited-resource conditions. To summarize, the HS-DNA extraction method is a cost-effective alternative and can be implemented with an on-site LAMP assay for the detection of *C. perfringens*.

Key Words: sensitivity; clostridium perfringens; dna extraction; LAMP assay; PCR assay

330P Mini-guts in a petri dish: development of long-term three-dimensional turkey intestinal organoids. Olivia Kibbey^{1*}, Marie Muffoletto¹, Dongmei Zhang¹, Paul DeFigueiredo¹, Morgan Farnell¹, Yuhua Farnell¹, ¹Texas A&M University, College Station, Texas, United States.

The digestive tract of poultry is essential for maintaining homeostasis within digestion, nutrition, and immune system elements. Unlike the study of human medicine, poultry must be euthanized in large quantities to study the effects of different conditions (nutrients, dietary supplements, and pathogens). By establishing a turkey intestinal epithelial cell model in vitro, we can reduce the number of poultry needed for testing and scientific research, implementing the principles of the 3Rs- replacement, refinement, and reduction. We hypothesize that given the proper environment and nutrition, it is possible to grow and sustain these cells collected from the duodenum of turkeys, animals for which this has not been done before. The objectives of this study were to: 1) develop ex vivo three-dimensional turkey intestinal organoids from crypt-containing stem cells and record the extent of organoid growth. 2) cryopreserve organoids. The duodenum houses a plethora of cells, including goblet cells, epithelial cells, Paneth cells, and stem cells. Stem cells located in crypts work to produce and replace damaged or dead cells by way of cell division. Currently, there are no turkey intestinal epithelial cell lines available for research. Culturing these stem cells from the duodenum would allow for the growth of duodenal cells and thus would allow for expanded research without the need to euthanize large numbers of poultry. The success of this experiment would allow for larger-scale studies in nutrient research, microbe interactions, and drug toxicology. Crypts were collected from the duodenum of 8 turkeys at embryonic day-23-old turkeys and seeded in Matrigel on a 24-well plate with a house-made organoid culture medium. The growth of live organoids was monitored for 4 days and images were captured using a Nikon Eclipse Ti microscope.

The statistical significance of differences in the size of the 4 turkey organoid replicates were analyzed by one-way ANOVA using JMP14. The duodenum organoid grew efficiently, increasing the average size of diameter three-fold in 4 days ($P < 0.05$). The organoids were successfully cryopreserved and could be passaged after being cryopreserved for 8 months. Successful cryopreservation was determined by effective thawing and growth of preserved cells. We are the first group to successfully establish three-dimensional organoids from turkey duodenum crypts, which can be cryopreserved and passaged for long-term use. The turkey's small intestinal organoids are a major step toward reducing reliance on animals in studying intestinal diseases, dietary supplement screening to improve gut health, and the developing of new vaccines for turkey.

Key Words: turkey; stem cell; duodenal intestine; crypt; organoid

331P Developing a simple method to make *Bacillus subtilis* competent cells with highly efficient electroporation transformation. Tahrir Alenezi^{1*}, Ying Fu¹, Ayidh Almansour¹, Hong Wang¹, Xiaolun Sun¹, ¹University of Arkansas, Fayetteville, Arkansas, United States.

Bacillus subtilis is an important platform for molecular biology engineering, especially for secretory enzymes. However, cloning and transforming DNA into *B. subtilis* are not as easy as *Escherichia coli*. For the transformation of *B. subtilis*, here we developed a new protocol to prepare super competent cells with high transforming efficiency. *B. subtilis* strain 168 cells were prepared for competent cells using four methods/protocols. In the first method of the traditional protocol, *B. subtilis* was grown in LB broth overnight to reach OD₆₀₀ of 0.9. *B. subtilis* was then diluted 16-fold in fresh 0.5 M sorbitol LB broth for 6 hr to reach OD₆₀₀ of 0.18. The *B. subtilis* cells were collected by centrifuging at 4000x g for 10 min. The cells were washed four times in ice-cold 0.5 M sorbitol and 0.5 M mannitol solution. The cells at 60 µl were immediately electroporation-transformed with 100 ng plasmid pDR111 or stored in 10% glycerol LB broth at -80 °C. The transformed cells were grown in 0.5 M sorbitol and 0.38 M mannitol LB broth for 3 hr. The cells were centrifuged, and the pellet was plated on 100 µg/ml spectinomycin LB selective plates. In method 2, the stored cells were transformed with 100 ng pDR111, recovered in the sorbitol and mannitol LB broth for 3 hr, and selected on the spectinomycin LB plates. In method 3, *B. subtilis* 168 cells were grown in 0.5 M sorbitol LB plate overnight and scrapped into LB broth reaching OD₆₀₀ of 1.7. *B. subtilis* was frozen in 10% glycerol LB or diluted 16-fold in fresh 0.5 M sorbitol LB broth for 6 hr at OD₆₀₀ of 0.6. The *B. subtilis* cells were pelleted, washed four times, transformed with 100 ng pDR111, recovered in the sorbitol and mannitol LB broth for 3 hr, and selected on the spectinomycin LB

plates. In method 4, 100 µl *B. subtilis* stock from method 3 was plated on a 0.5 M sorbitol LB plate for 6 hr. The *B. subtilis* cells were scrapped, washed four times, transformed with 100 ng pDR111, and recovered in the sorbitol and mannitol LB broth for 1 hr, and one-tenth of the volume was plated on the spectinomycin LB plates. The shape of *B. subtilis* cells was visualized by microscope. Notably, *B. subtilis* cells in methods 1 and 2 were longer than the cells in methods 3 and 4. The transformation efficiency was 50, 0, 1.02×10^3 , 1.25×10^5 transformants/µg of pDR111 DNA for method 1, 2, 3, and 4, respectively. In conclusion, we developed a simple *B. subtilis* competent cell preparation method with transformation efficiency thousand folds higher compared to the traditional method. The finding will help the molecular biology field using the *B. subtilis* platform.

Key Words: *B. subtilis*; transformation; sorbitol; mannitol

332P Melanocortin 1 Receptor (MC1R) gene variants-based analyses of relationships among turkeys. Edward Smith^{1*}, J. M. B. Adikari², ¹Virginia Tech, Blacksburg, Virginia, United States, ²Rajarata University of Sri Lanka, Mihintale, Sri Lanka.

Heritage turkeys provide unique resources for the genetic improvement of commercial birds. Our understanding of their relatedness to the commercial turkey, thus their value for genomic improvement remains very limited. Here, we evaluated the distribution of haplotypes identified using oligos specific for the turkey Melanocortin 1 Receptor (MC1R) gene among 5 heritage turkeys including Blue Slate (n=40), Broad Breasted Bronze (n=48), Royal Palm (n=45), Spanish Black (n=48), Midget White (n=40) and a commercial population (n=40). The birds were obtained from commercial sources and maintained at Virginia Tech for previous investigations. Validated genomic information from a total of 252 birds were included in the haplotyping based on 9 SNPs and 7 distinct and validated haplotypes. The validation was based on resequencing the opposite strands as well as chi squared test of the deviation from expected within each strain. Freely available web-based software, Genome Variation Server (<https://gvs.gs.washington.edu/GVSBatch150/HelpAbout.jsp>), was used in the haplotype count and analyses. Two haplotypes, Hap04 and Hap07, were the most frequent. Though uneven, all the heritage strains and the commercial turkey shared all 7 haplotypes. Unlike chickens, as has been previously reported, our data suggest that the *MC1R* polymorphism may not be useful in distinguishing among turkey strains. The data does not support our earlier observations, based on other genetic tools including microsatellites, that the Royal Palm is not a distinct a heritage strain.

Key Words: Haplotypes; Turkeys ; SNPs; heritage birds

333P Innovative strategies to understand the killing mechanism of Peracetic acid against *Salmonella*

***Typhimurium* 14028s in poultry products and food industry and its usage impact on the development of Multi-Drug Resistant strains.** Mohammed Aldoury^{1*}, ¹University of Arkansas, Fayetteville, Arkansas, United States.

Peracetic acid is one of the most common bactericidal agents used in poultry products, nevertheless, its killing mechanism has not been understood yet. Hence, there is a need to find new strategies to understand its mechanism of action and to enhance its antimicrobial efficiency. Previous research has shown that the killing mechanism of Peracetic acid involve Reactive Oxygen Species (ROS) production. We believed that enhancing ROS production inside bacterial cells will increase antimicrobial activity. In our study, we demonstration that ROS production can be predictably improved in *Salmonella Typhimurium* 14028s by deleting the genes that contribute to the cell's capacity on decreasing ROS production, which increases the susceptibility of bacterial cells to oxidative stress. In order to demonstrate that, different *Salmonella typhimurium* 14028s mutant strains with single deleted gene were used to estimate mutation rate and the ROS production after exposure to sublethal concentration of peracetic acid. This study creates a schematized assay to modify ROS production in bacteria and proves that increased bacterial ROS production can strengthen killing efficiency by sanitizing antimicrobial agents and antibiotics.

Key Words: Peracetic acid; mechanism

334P Effects of dietary supplementation of Magni-Phi® Ultra on intestinal permeability and immune response in broiler chickens during a coccidia challenge. Saheed Osho^{1*}, Kari Saddoris-Clemson¹, Brooke Humphrey¹, Miriam Garcia¹, ¹Phibro Animal Health Corporation, Corvallis, Oregon, United States.

Poultry coccidiosis causes severe damage to intestinal tissue and induces a mucosal immune response. Magni-Phi Ultra (MPU; Phibro Animal Health, Teaneck, NJ) contains a highly concentrated source of natural saponins from *Yucca schidigera* and *Quillaja saponaria*. Saponins exert numerous biological activities, with specific toxicity to protozoans as related to their detergent effect on the cell membranes. This may provide benefit to the commercial poultry industry to reduce pathogen load in antibiotic-free poultry production systems. The objective was to determine the effects of MPU supplementation on intestinal integrity and immunity in broiler chickens challenged with a mixed coccidia infection during peak {d 0 to d 5 post-challenge (PC)} and recovery phases (d 5 to d 12 PC). A total of 128 male, 6-d-old Ross 708, broiler chicks were allotted to 4 treatment groups (8 replicate cages; 4 birds/cage) in a randomized complete block design. Treatments included a non-challenge positive control (POS), a cocci challenge (CC) negative control (NEG), CC fed salinomycin (66 ppm: SAL), and CC fed MPU (0.11 g/kg of diet; MPU). Diets were corn-soybean meal-based in mash form. At 16 days

post-hatch, all birds except for POS were orally gavaged with 25 doses/kg BW of a coccidia vaccine. On d 5 and 12 PC, 2 birds/cage were orally gavaged with fluorescein isothiocyanate conjugate dextran (FITC-D) and blood and jejunum were collected 2 h later. On d 5 PC, the duodenum was evaluated for lesions and enumeration of oocysts per gram of excreta (OPG). Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were calculated over the experimental period. Data were analyzed with GLIMMIX procedure of SAS. On d 5 PC, NEG birds had reduced BWG (21%), a 2-fold increase in serum FITC-D, and increased lesion scores and oocyst shedding compared to POS birds ($P < 0.05$). Birds in both MPU and SAL treatments showed increased BWG, decreased lesion scores and oocyst shedding, and reduced plasma FITC-D compared to NEG birds ($P < 0.05$). On d 5 PC jejunum IL-17, IL-10, and IFN-gamma mRNA expression were higher in NEG compared to POS ($P < 0.05$) while both MPU and SAL were similar to POS ($P > 0.05$). On d 12 PC, NEG birds had increased plasma FITC-D and higher jejunum IL-10 and IFN-gamma mRNA expression compared to POS birds ($P < 0.05$). Both MPU and SAL birds had lower plasma FITC-D and jejunum IL-10 and IFN-gamma mRNA expression compared to NEG birds ($P < 0.05$) but similar to POS ($P > 0.05$). This study confirms that MPU improves intestinal integrity and mucosal immune responses during peak and recovery phases following a mixed coccidiosis challenge.

Key Words: coccidiosis; cytokines; Broiler chickens; intestinal permeability; Magni-Phi Ultra

335P Evaluation of post-challenge *Salmonella* Enteritidis colonization in commercial layers fed R2, an energy-based additive. Abdullah Mahfuz^{1*}, Matthew Jones², Mohan Dasari¹, Roy Berghaus³, Charles Hofacre², ¹Feed Energy Company, Des Moines, Iowa, United States, ²Southern Poultry Research Group, Inc, Watkinsville, Georgia, United States, ³The University of Georgia, Athens, Georgia, United States.

Salmonella Enteritidis (S.E.) is one of the leading causes of acute bacterial human gastroenteritis from poultry. R2 is a lipid-based product containing a blend of short, medium, and long chain fatty acids with high oxidative stability. The research objective was to evaluate efficacy of R2 on S.E. tissue colonization in commercial layer pullets after an acute challenge. Each dietary treatment was represented by 48 one-day-old Hy-Line W-36 layer pullets. The control group was fed distillers corn oil (DCO) at an inclusion of 2.5% from day of placement to 12 weeks of age and a diet containing 1.0% DCO from 12 to 20 weeks of age. The R2 treatment was included at the same rate as distillers corn oil during these intervals. Diets were prepared using basal diet manufacture to ensure only the oil components were different between groups. At 10 weeks of age, pullets were placed in individual cages with a plastic wall between the R2 treatment and the challenge control. The day length was

increased 1 hour each week to 16 hours to stimulate ovary development. All birds were orally gavaged S.E. at 17 weeks with 5.2×10^8 CFU/bird. Ceca and ovaries were collected from half of the birds in each treatment on week 18 and from the remaining birds on week 20 (1- and 3-weeks post infection, respectively). Cloacal swabs were collected 3 days and 2 weeks post infection. S.E. prevalence in ceca, ovaries, and cloacal swabs was compared between treatments and weeks using logistic regression. *Salmonella* CFUs in culture-positive samples were compared using linear regression. All statistical testing assumed a two-sided alternative hypothesis, and $P < 0.05$ was considered significant. While there were no statistical differences in tissue colonization, there was consistency in the direction of the data from each tissue. Overall, the birds fed R2 had numerically lower S.E. prevalence in ceca (79%^a of S.E. positive versus 66%^a in the R2 treated hens)($P=0.106$). The number of S.E. positive ovaries in the control pullets increased from week 18 (17%) to week 20 (26%) where the R2 treated ovaries declined from 21% to 9% over the same period. At 20 weeks, there was a trend in decreased ovary prevalence ($P=0.110$). *Salmonella* prevalence from cloacal swabs were consistent with the ovary results. Prevalence of each tissue was lower at 20 weeks in the treated group indicating R2 may have long term benefits in preventing and/or reducing S.E. colonization of the ceca and ovaries.

Key Words: Salmonella; Antimicrobial; Additive; Layer Pullets; Low-pKa based fat

336P Impact of dietary supplementation of Provia Prime™ on intestinal permeability during a coccidia challenge and feed withdrawal recovery in broiler chickens. Saheed Osho^{1*}, Kari Saddoris-Clemmons¹, Brooke Humphrey¹, Miriam Garcia¹, ¹Phibro Animal Health Corporation, Corvallis, Oregon, United States.

Maintaining intestinal integrity supports optimal gut functionality and influences overall performance of broiler chickens. Provia Prime (PP) contains a unique combination of four probiotic strains of *Bacillus spp* selected to promote a healthy gut and improve performance. Two experiments (Exp) were conducted to determine the effects of PP supplementation on intestinal permeability (IP) during a coccidia challenge (CC) and feed withdrawal (FW) model. Diets in both Exp were corn-soybean meal-based in mash form. Intestinal permeability was assessed by orally gavaging birds with 8.4 mg fluorescein isothiocyanate conjugated dextran (FD4) per kg BW. Blood samples were collected 2h post-FD4 administration and plasma was analyzed for FD4. In Exp 1, 7-d old Ross 708 male broiler chicks (n=64) were allotted to 1 of 4 treatment (Trt) groups (8 cages/trt; 2 birds/cage) in a randomized complete block design. Treatments included: non-challenge negative control (NEG), CC control (cPOS), CC fed salinomycin (66 ppm: SAL), and CC fed PP (500,000 CFU/g of diet). All birds except NEG were orally gavaged on d16 post hatch

with 3× the recommended coccidia vaccine (Coccivac B52®) using 25 doses/kg BW. On d5 post-challenge (PC), IP was measured, excreta samples were collected for enumeration of oocysts per gram of excreta (OPG) and birds were euthanized for scoring of coccidia-induced duodenal lesions (LS). Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were calculated during the challenge period. In Exp 2, 9-d old Ross 708 male broiler chicks (n=96) were randomly allotted to 1 of 3 trts (8 cages/trt; 4 birds/cage). Treatments included: a non-FW group, a FW positive control (fPOS), and FW fed PP (500,000 CFU/g of diet). All birds except those in non-FW group had feed withdrawn on d21 for 10h to induce intestinal dysbiosis and feed was placed back after 10h FW for 2 days. After 10h of FW, IP was measured from 2 birds/cage. Two days post 10h FW, IP recovery was measured from the remaining birds. Data were analyzed with GLIMMIX procedure of SAS. In Exp 1, cPOS birds had 27% reduction ($P<0.05$) in BWG, and a 2.6-fold increase ($P<0.05$) in plasma FD4 when compared to NEG. Birds in both PP and SAL groups showed increased ($P<0.05$) BWG and decreased ($P<0.05$) OPG, LS and plasma FD4 when compared to cPOS birds. In Exp 2, after a 10h feed withdrawal, fPOS birds had a 2.7-fold increase ($P<0.05$) in plasma FD4 when compared to non-FW birds but birds fed PP showed decreased ($P<0.01$) FD4 as compared to fPOS. After a 2-d recovery, PP-fed birds had similar plasma FD4 when compared to non-FW birds. This study confirms that PP improves intestinal integrity during a coccidia challenge and in a feed withdrawal model.

Key Words: Broiler chickens; intestinal permeability; coccidia challenge; Feed withdrawal; Provia Prime

337P Evaluation of the impact of mycotoxins on the incidence of bacterial chondronecrosis with osteomyelitis in broiler chickens. Adnan Alrubaye¹, Amer Hasan¹, Khawla Alharbi^{1*}, Abdulkarim Shwani¹, Sonali Lenaduwe Lokuge¹, Kyle burks¹, Douglas Rhoads¹, Raj Murugesan², Shelby Ramirez², ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Head - Global Poultry at DSM, West Des Moines, Iowa, United States.

Bacterial Chondronecrosis with Osteomyelitis (BCO) lameness is the most critical animal welfare issue facing the poultry industry resulting in significant economic losses. BCO lameness is a metabolic disease that affects fast-growing birds and is caused by various pathogenic microorganisms like *Staphylococcus*, *Escherichia coli*, and *Enterococcus* species. Our research group uses two models to induce BCO: bacterial water challenge using *Staphylococcus agnetis* and physical stress using wire floor. As a result of previous projects, we discovered that the wire-flooring system radically alters the structural integrity of the small intestine. Mycotoxins (MTX) reduce the barrier strength of the intestinal tract and have immunosuppressive effects, and are found in corn and soybean meal diets as deoxynivalenol, zearalenone, and

fumonisin. Therefore, this study aimed to evaluate the impact of adding mycotoxins to the diet of broiler chickens raised on litter when the infection source is birds raised on the wire floor. We examined two dietary MTX contamination levels provided by Biomin, a low level of MTX (L-MTX) and a high level of MTX (H-MTX), with two types of flooring, flat-wire (WF) and fresh-litter (FL). We used 720 Cobb500 male chicks assigned to 1 of 4 treatments in a 2 * 2 factorial design. This study used six pens per treatment and 30 birds per pen from days 0 - 14, and 25 birds per pen from days 14 - 57. BCO was quantified by clinically observing lame birds and necropsying them for lesions. The cumulative incidence of lameness per treatment was recorded, including scoring of lesions of tibial and femoral lesions. On d 42 and 56, intestinal permeability was assessed via recovery of fluorescein isothiocyanate dextran (FITC-d; 3 – 5 kDa) marker from serum 1-hour post orally gavage with 8.32 mg FITC-d/kg BW, and samples were transferred to the lab and measured directly. Mortality and BCO lameness percentages were analyzed with the GLIMMIX procedure of SAS. A higher percentage of birds on H-MTX (70.4%) displayed lameness than those on L-MTX (52.8%), and a higher rate of birds on WF (74.7%) showed lameness than those on FL (47.5%). A significant percentage of BCO lameness was observed with H-MTX on FL (+36%) than WF (+15%). However, FITC-d recovery in sera was more significant ($P<0.05$) in birds on litter-floor compared with birds on the wire floor on d 42 (FL 43.4 ng/ml, WF 14.0 ng/ml) and d 56 (FL 10.9 ng/ml, WF 0.4 ng/ml). Bacteria commonly present were *E. faecalis*, *Staphylococcus* spp, and *E. coli*. Our results suggest that higher levels of MTX in the diet are associated with increased intestinal permeability and a decrease in intestinal barrier integrity, thus causing increased BCO lameness.

Key Words: Lameness; Mycotoxin; Chondronecrosis; Intestinal permeability; Osteomyelitis

338P Natural antibody response to vaccination in poultry. Kendra Chambliss^{1*}, Kellen Pautzke¹, Michael konkel¹, Kayla Niel², Janet Fulton², Jeb Owen¹, ¹Washington State University, Pullman, Washington, United States, ²Hy-Line International, Dallas Center, Iowa, United States.

Natural antibodies (**nAbs**) are a category of immunoglobulins that are present before infection as part of the innate immune system. Levels of nAbs are treated as reliable indicators of immunological competence that are independent of the adaptive immune response (**AR**). It is unclear how nAbs interact with the AR and some evidence suggests that nAbs change after immune challenge. To evaluate the relationship between nAbs and the AR, we conducted experiments to explicitly test how nAbs respond to immune challenge. In Experiment 1 we used 144 chickens representing 6 different breeds that were divided into vaccinated and unvaccinated groups (12 birds/vaccination treatment/breed). Vaccinated chicks were

injected with a *Campylobacter jejuni* whole cell lysate (WCL) at 1 and 2 wks of age. Serum was collected after euthanasia at 4 wks of age. In Exp 2, serum was collected from 100 chickens representing two breeds (50 birds/breed) before and after vaccination against Fowl Pox virus (FP), Avian Encephalomyelitis Virus (AEV), and Chicken Anemia Virus (CAV). Natural and FP antibodies were measured using custom sandwich ELISAs that detected antibodies binding to keyhole limpet hemocyanin (KLH), ovalbumin (OVA), and FP proteins. Induced antibodies for AEV and CAV were measured using commercial ELISA kits (BioChek, Maine, U.S.). Generalized mixed effect models were used for statistical analyses for all experiments. A significance level of <0.05 was used. In Exp 1, higher levels of antibodies against *C. jejuni* were observed among all vaccinated birds compared to unvaccinated birds. The nAbs binding KLH and OVA also increased in birds vaccinated with *C. jejuni* compared to non-vaccinated birds. Breed affected both nAb and induced antibody levels. In Exp 2, antibody levels were compared pre- versus post-vaccination. Antibodies against FP, AEV, and CAV increased after vaccination and antibody levels differed among breeds. Pre-vaccination nAb levels were not correlated with post-vaccination antibodies against any virus, indicating nAbs had no detectable effect on induced antibodies. The nAb levels increased post-vaccination and levels of nAbs were affected by breed. We found no evidence for an effect of nAbs on the magnitude of the induced antibody response (Exp 2). However, nAbs increased in vaccinated chickens in both experiments, indicating that nAbs are not independent of the AR. The AR appears to affect production of nAbs, but it remains unclear if nAbs affect the adaptive response. Finally, we found evidence of breed effects on both nAb and induced antibody expression that underscores the importance of genetic variation to our understanding of antibody function in chickens.

Key Words: poultry; Vaccination; natural antibody; innate immune; adaptive immune

339P Effects of necrotic enteritis on growth performance, intestinal health, and bone quality in two different chicken strains: Athens Canadian Random Bred 1955 (ACRB) and Cobb 500. Doyun Goo^{1*}, Dima White¹, Milan Sharma¹, Hanseo Ko¹, Janghan Choi¹, Woo Kim¹, ¹University of Georgia, Athens, Georgia, United States.

The aim of the current study was to investigate the effects of necrotic enteritis (NE) models on growth performance, intestinal health, and bone quality in two different chicken strains, Athens Canadian Random Bred 1955 (ACRB) and Cobb 500. The study was conducted in a completely randomized design with a 2 x 3 factorial arrangement consisted with 2 strains and 3 different coinfection disease model using *Eimeria maxima* orally infected on d 14 and *Clostridium perfringens* on d 18 (4 days post infection: dpi). A total of 360 1-d-old male (180 ACRB and 180 Cobb

500) chickens were allotted to 6 groups with 6 replicates. Within each chicken strain, birds were allocated to non-challenged control (NC) and 2 different *E. maxima* oocysts of 2,500 and 12,500 + 1 x 10⁹ *C. perfringens* (2,500⁺ and 12,500⁺). The experiment lasted for 25 days and chickens were euthanized on d 20 and 22 (6 and 8 dpi) for the analyses of intestinal permeability and lesion scores and d 25 (11 dpi) for bone mineral density (BMD) and bone mineral contents (BMC) using DEXA. All chickens were raised according to the Cobb 500 management guide, and dietary feed was mixed based on the nutrient requirements of Cobb 500. All data were statistically analyzed by 2-way analysis of variance using software RStudio, and the relative body weight (RBW), relative body weight gain (RBWG), relative feed intake (RFI), and relative feed conversion ratio (RFCR) were analyzed by setting the NC treatment to 100%. Results showed that there was an interaction ($p < 0.05$) on RBWG of d 14 to 20 and 22 between strains and NE models because decreased RBWG more in the ACRB with increased dosages of *E. maxima* than the Cobb 500. Chickens in 12,500⁺ treatment decreased RBW compared to the other treatments at d 20 and 22 ($p < 0.001$), and RFI and RFCR also decreased compared to chickens in the NC ($p < 0.01$). There were interactions ($p < 0.05$) for intestinal permeability using FITC-dextran on 6 and 8 dpi between strains and NE models because chickens of the ACRB dramatically increased intestinal permeability compared to the Cobb 500 as the challenge dosage increased. Jejunal lesion scores of 6 dpi showed an increased lesion scores in chickens of 12,500⁺ treatment compared to NC and 2,500⁺ treatments ($p < 0.001$), but there was no statistical difference in 8 dpi. Bone quality results showed that BMD and BMC of Cobb 500 were increased compared to those of the ACRB ($p < 0.001$), and BMC was decreased in treatment 12,500⁺ than NC regardless of strain ($p < 0.05$). In conclusion, NE negatively affects growth performance, intestinal health, and BMC regardless of chicken strain, and ACRB are relatively more vulnerable than Cobb 500 under NE challenged conditions.

Key Words: Necrotic enteritis; Broiler; Intestinal health; Bone quality; Athens Canadian Random Bred

340P H. meleagridis inoculation decreases the immunocompetence of turkey poults seven days after challenge. Jun Yang^{1*}, Katherine Cupo¹, Frank Eden¹, Chongxiao Chen¹, ¹North Carolina State University, Raleigh, North Carolina, United States.

Histomoniasis, an infectious disease caused by the protozoan parasite *Histomonas meleagridis*, has devastating impacts on the health and welfare of infected flocks, especially in commercial turkey industries. Few studies have been performed to understand the immune-related interactions between the protozoan and host cells. The goal of this study was to characterize the cellular immune function of turkey poults after they were infected with *H. meleagridis*. 60 one-day-old poults were placed in 6

isolation cages with 10 poult per cage. At 14 days of age, half poult (3 cages x 10 poult) were intra-cloacal inoculated with 100,000 *H. meleagridis* cells. The phytohemagglutinin (PHA) skin swelling test was conducted to investigate the effects of *H. meleagridis* infection on the immunocompetence of turkey poult. At 6 days after *H. meleagridis* inoculation, all 60 poult received an intradermal injection of 100 mg of PHA-P in 100 µl of SBS into the wing web. The index of stimulation, a metric used to compare the degree of inflammation induced by PHA-P injection, was calculated for each bird based on the difference in wing web thickness measured just prior to PHA-P injection and 38 hours after injection. The trial was repeated twice with the same procedure. All birds were raised to 4 weeks of age and necropsied to examine the ceca and liver to confirm *H. meleagridis* infection in inoculated birds based on the presence of macroscopic pathology. Using the isolation cages as the experimental units and the birds as the observational units, statistical analysis of the index of stimulation between treatments were performed using the GLIMMIX procedure in SAS v9.4 (SAS Institute, Cary, NC, USA). The statistical model included treatment and trial main effects as well as the treatment x trial interaction. Results were considered significant with $P \leq 0.05$. The results show no interaction between treatment and trial ($P=0.9819$). The treatment's main effect demonstrated that infected poult had a lower index of stimulation compared to non-inoculated poult ($P=0.0080$). This indicates that *H. meleagridis* infection decreases the inflammatory response to PHA. From the trial main effects, it was evident that the index of stimulation in Trial 2 was higher than in Trial 1 ($P < 0.0001$), which may be attributed to the use of two different types of calipers in either trial. This study demonstrates that *H. meleagridis* infection in turkeys negatively affects immunocompetence as determined by PHA skin swelling analysis. This decreased immunocompetence could leave the birds vulnerable to subsequent infections.

Key Words: turkey; phytohemagglutinin (PHA); immunocompetence; *Histomonas meleagridis*; Histomoniasis

341P Dietary lactobacillin supplementation on growth performance, antioxidant capacity and intestinal health of broiler challenged by *Clostridium perfringens*. Lei Yan^{1*}, Sha An^{1,2}, Dan Liu⁴, Zunzhou Lv^{1,2}, Mingbin Lv¹, Jianshu Zhuo¹, Xiaoguo Lv^{1,2}, Yong Li^{1,2}, Kun Xing¹, Yuming Guo⁴, Guilian Zhou^{1,2}, ¹Shandong New Hope Liuhe Group Co., Ltd., Qingdao, China, ²New Hope Liuhe Co., Ltd., Beijing, China, ³Key Laboratory of Quality Control for Feed and Products of Livestock and Poultry, Ministry of Agriculture, Chengdu, China, ⁴China Agricultural University, Beijing, China.

This study investigated the effects of lactobacillin on growth performance, immunity, antioxidant capacity, intestinal health and microflora of broilers infected

with *Clostridium perfringens*. A total of 320 one-day-old male AA broilers randomly divided into 4 groups (negative control group, NC; positive control group, PC; antibiotic group, AGP; lactobacillin group, Lac) with 8 replicates and 10 broilers in each replicates. All the broilers in the four groups were fed with basal diet, of which the AGP were treated with 10 mg/kg of enramycin, LAC were treated with 0.5% lactobacillin. During 14-20d, PC, AGP and Lac group were challenged with *Clostridium perfringens*. Higher body weight (21d and 42d), ADFI and ADG (22-42d, 1-42d) and lower FCR (1-42d) were observed in NC group compared with PC group ($P < 0.05$). Both antibiotics and lactobacillin could decrease the FCR and increase the ADG in 22-42d and 1-42d ($P < 0.05$). The addition of lactobacillin tended to increase the serum T-SOD in 21d ($P=0.084$). The serum MDA level of broilers in Lac group was significantly decreased in 42d ($P < 0.05$). Lac group had a higher IgG levels in 21d ($P < 0.05$). Compared with the NC group, challenge led to a significant reduction in villus height ($P < 0.05$), and the addition of lactobacillin not only improved villus height ($P < 0.05$) but also had a trend to alleviate intestinal damage ($P=0.06$). The challenge resulted in a increased intestinal microflora diversity, decreased abundance of *Firmicutes* and increased abundance of *Vibrio butyricum* ($P < 0.05$). Lac group has a higher abundance of *Firmicutes*, *Bacteroides* and lower diversity ($P < 0.05$). In conclusion, the addition of lactobacillin in broiler diets can improve the growth performance, relieve intestinal damage caused by *Clostridium perfringens* challenge, enhance antioxidant capacity and immune function, and improve the structure of intestinal microflora.

Key Words: broilers; *Clostridium perfringens*; growth performance; lactobacillin; intestinal flora

342P Changes in percentages of circulating leukocytes of *Histomonas meleagridis*-infected turkeys. Katherine Cupo^{1*}, Javid Mohammed², Frank Edens¹, Chongxiao Chen¹, ¹North Carolina State University, Raleigh, North Carolina, United States, ²Duke Cancer Institute, Durham, North Carolina, United States.

Histomoniasis is an infectious disease caused by *Histomonas meleagridis* that produces high mortality in turkeys. No countermeasures are available, and little is understood about the immune cascade initiated by *H. meleagridis*. Many immune cells may be involved in this cascade, most of which may be found circulating in the blood. Therefore, the objective of this study was to characterize the cellular immune response in the blood of turkeys at the height of infection, 7 days after inoculation with pathogenic *H. meleagridis*. Two experiments were performed. In both experiments, 20 poult were placed into 2 isolation cages, 10 poult/cage, and the poult in 1 cage were inoculated with 100,000 *H. meleagridis* cells/bird at 2 weeks of age. Seven days later, blood was collected from all poult to quantify T-cell subsets (primary components of

adaptive immunity) using flow cytometry (experiment 1) or prepare Wright-Giemsa-stained blood smears to quantify other leukocytes (experiment 2). Cells isolated from blood samples used for flow cytometry analysis were stained with fluorescently labeled antibodies to separate T-cells (CD3⁺) from all other leukocytes and to distinguish helper (CD4⁺CD8⁻), cytotoxic (CD8⁺CD4⁻), and regulatory (CD4⁺CD25⁺CD8⁻) T-cell subsets. From the blood smears, a cumulative total of 100 lymphocytes, heterophils, basophils, eosinophils, and monocytes were counted per smear. Percentages of T-cell subsets (experiment 1) or leukocytes (experiment 2) were compared between infected and non-inoculated poulters using the GLIMMIX procedure in SAS v9.4 with treatment fixed effect and LSMEANS separation using the PDIF option. The results from experiment 1 demonstrated that, within CD3⁺ T-cells, the percentage of CD8⁺CD4⁻ cytotoxic T-cells is significantly decreased ($P = 0.0041$) while the percentage of CD8⁺CD4⁺ double positive T-cells is significantly increased ($P = 0.0102$) in response to *H. meleagridis* infection. No other T-cell subset was significantly altered. It was also observed based on forward and side scatter parameters that the percentage of granulocytes was greater ($P < 0.0001$) while lymphocytes was lower ($P < 0.0001$) in infected poulters. The results from experiment 2 similarly demonstrated that the percentage of lymphocytes decreased ($P < 0.0001$) while the percentage of heterophils (a granulocyte) increased ($P = 0.0001$) in response to *H. meleagridis* infection. The percentages of all other leukocytes were not significantly altered. Because elevated levels of heterophils in the blood are indicative of a response to damage as opposed to infection, this study indicates that turkeys are responding to tissue damage caused by *H. meleagridis* infection at 7 days post-inoculation.

Key Words: *Histomonas meleagridis*; flow cytometry; T-cells; granulocytes; heterophils

343P Searching for a cure for *Cochlosomosis*: A meta-analysis of oral supplementations on reducing the *Cochlosoma anatis* in turkeys. Christina Sigmon^{1*}, Jinquan Wang², Harshavardhan Thippareddi², Robert Beckstead³, Chongxiao Chen¹, ¹*North Carolina State University, Raleigh, North Carolina, United States*, ²*University of Georgia, Athens, Georgia, United States*, ³*Ceva Animal Health, Lenexa, Kansas, United States*.

Cochlosoma anatis is a flagellated protozoan that has been associated with enteritis, stunting, and early mortality in turkeys. Currently, there is no treatment available. An infection model was used in our lab to screen potential oral supplements on the effectiveness of reducing *C. anatis* infection in turkeys. In this research model, turkey poulters were orally inoculated with 500,000 *C. anatis* cells/bird at ~2 weeks of age. At week 4 of age, birds were euthanized for the level of *C. anatis* in 7.5 cm of the small intestine downstream from Meckel's diverticulum

using a Neubauer hemocytometer to count the number of parasites. Since 2019, we have screened products from 10 companies, and tested 14 products in 6 categories (Medication, Organic acid, Plant extracts, Prebiotic/probiotic/postbiotic, Prototype, and Mixture: with two or more compounds mentioned above). 5 products were tested at different levels. A total of 23 trials were conducted in our lab. The *C. anatis* count in milliliters was log 10 transformed. Data analysis was performed using R version 4.0.2 (R Core Team, 2021). A linear regression model was built using lm function in R to evaluate the correlation of the oral supplement type and *Cochlosoma* counts (log cells/ml). From the result, Medication category increased the count by 0.37 log cell/ml compared to control ($P < 0.05$). Meanwhile, supplementation of Prebiotic/probiotic/postbiotic showed a trend to increase the cell count by 0.22 log cells/ml ($P < 0.1$). However, supplementation of Mixture could reduce the cell count by 0.67 log cells/ml compared to control ($P < 0.05$). Supplementation of Plant extracts also showed a trend to reduce the cell count by 0.16 log cells/ml ($P < 0.1$). Meanwhile, the pooled results from different categories with various doses suggested there is no general dose effect on cell count. In summary, Mixture and Plant extracts tested in our lab showed a higher potential in reducing *C. anatis* in turkeys.

Key Words: meta-analysis; turkeys; *Cochlosoma anatis*; oral supplements; infection model

344P Effects of Clarity Q on mRNA abundance of nutrient transporters during a subclinical necrotic enteritis. Candice Blue^{1*}, Nima Emami¹, Omar Gutierrez², Staci Cantley², Rami Dalloul¹, ¹*University of Georgia, Athens, Georgia, United States*, ²*Huvepharma, Morgantown, West Virginia, United States*.

During subclinical necrotic enteritis (NE), damage to the gastrointestinal epithelial layer leads to reduced digestion and absorption of nutrients, which negatively affects birds' performance. Utilizing phytogetic feed additives could mitigate intestinal damage and improve nutrient absorption, thus enhancing the bird's health status. This study evaluated the effects of Clarity Q (CQ) on mRNA abundance of nutrient transporters in the jejunum during a NE challenge. A total of 900 day (d)-old male broilers were allocated to three treatments with 10 replicate floor pens (30 birds/pen) as follows: 1) negative control (NC): birds fed a corn-soybean meal diet; 2) positive control (PC): NC + 50 g/metric ton (MT) BMD; and 3) herbal extract (CQ15; Clarity-Q: NC + 15 g/MT CQ. A naturally occurring NE model was induced by spraying coccidiosis vaccine (10× Advent) onto the feed and litter right before bird placement coupled with *C. perfringens* spores located in the barn. On d 8 (at peak NE) and d 14 (post-NE recovery), one bird/pen (n=10) was necropsied and jejunal samples were collected. The mRNA abundance of peptide transporters (PepT)-1 and PepT2, excitatory amino acid transporter (EAAT)-3,

sodium-dependent glucose cotransporter (SGLT)-1, glucose transporter (GLUT)-2, and GLUT5 were measured. Data were analyzed using ANOVA (JMP, Pro 16) and significance ($P \leq 0.05$) between treatments was determined by the LSD test. On d 8, mRNA abundance of EAAT3, GLUT2, and PepT2 were significantly greater in CQ15 birds compared to those in NC but not PC birds. PC birds had significantly greater mRNA abundance of GLUT5, PepT1, and SGLT1 compared to CQ15 and NC. On d 14, mRNA abundance of EAAT3, GLUT5, and PepT1 was not statistically different between the treatment groups. In contrast, the mRNA abundance of GLUT2, PepT1, and SGLT1 in the CQ15 birds was similar to those in NC. Based on previously reported performance data and the current results, an upregulation of nutrient transporters EAAT3, GLUT2, PepT1, and PepT2 correlates with the improvement of body weight gain, reduced lesion severity, and modification of tight junction proteins when birds were supplemented with Clarity Q. Higher abundance of these transporters may translate into enhanced absorption and subsequently better utilization of nutrients, potentially alleviating the negative impacts caused by this enteric disease.

Key Words: broiler; *C. perfringens*; nutrient transporter; Necrotic enteritis; absorption

345P Investigation of virulence factors in avian pathogenic *Escherichia coli* isolated from asymptomatic and diseased broilers. Priyanka Devkota^{1*}, Sabin Poudel¹, Linan Jia¹, Xue Zhang¹, Anuraj Sukumaran¹, Aaron Kiess², Jeffrey Evans³, Pratima Adhikari¹, Li Zhang¹, ¹Mississippi State University, Mississippi State, Mississippi, United States, ²North Carolina State University, Raleigh, North Carolina, United States, ³USDA, Mississippi State, Mississippi, United States.

Virulence factors related to the pathogenicity of avian pathogenic *Escherichia coli* (APEC) perform a wide range of activities related to bacterial colonization, adhesion, iron acquisition, and invasion. This study aimed to investigate the genotypic and phenotypic attributions of underlying pathogenicity of APEC. A total of 66 *E. coli* isolates were collected, out of which 44 were obtained from disease-associated lesions, and 22 were collected from asymptomatic broilers and environmental samples from poultry farms. The five virulence genes (*iutA*, *iss*, *hlyF*, *ompT* and *iroN*) which are associated with pathogenic APEC strains were detected by multiplex polymerase chain reaction (PCR). Swarming motility was detected using 0.4% semi-solid LB agar which helps to determine the bacterial flagellar mobilization. Growth curve analysis was done using a microplate reader to observe the difference in growth patterns between asymptomatic and disease carrier strains. Statistical analysis was performed using the SAS 9.4 software with chi-square for virulence test, and a two-sided t-test for doubling time. Out of five virulence genes tested, *iss* (97.73% vs 72.73%) and *iroN* (95.45% vs

72.73%) were significantly higher in disease-associated strains than in asymptomatic carriers ($P \leq 0.05$). The disease-associated group had a greater proportion of hypermotile isolates in contrast to the asymptomatic group (34.09% vs 9.09%, $P \leq 0.05$). Additionally, 27.27% of asymptomatic carrier isolates were non-motile, which is higher compared to disease-associated strains (2.27%). The mean doubling time among disease-associated and asymptomatic strains was 32.05 and 35.51 minutes respectively ($P > 0.05$). A 12.45% decrease in doubling time was observed in disease-associated strains compared to the asymptomatic carrier strains. In conclusion, the data revealed that disease-associated strains showed a higher number of virulence genes than asymptomatic strains. Collectively, our data showed that the virulence factors and phenotypic characteristics of APEC may play a role in the pathogenesis of avian colibacillosis. Therefore, this study provides insight to understand the epidemiological background, microbial behavior, and pathogenesis of APEC.

Key Words: broiler; motility; growth curve; virulence; Avian pathogenic *Escherichia coli*

346P Protective immunity against recent field isolates of coccidia challenged broilers immunized with a live commercial vaccine and fed a phytogetic/probiotic blend product. Jennifer Timmons^{1*}, Celia Whyte¹, Steve Fitz-Coy², Vanessa Iseri³, ¹University of Maryland Eastern Shore, Princess Anne, Maryland, United States, ²Merck Animal Health, Salisbury, Maryland, United States, ³Kemin Industries, Inc, Des Moines, Iowa, United States.

A pen trial using 1,920 Ross 708 straight run broiler chicks (4 treatments with 8 replicates each) was conducted to measure the level of protection of a live commercial vaccine (LCV) when birds were fed a diet supplemented with a phytogetic and probiotic blend product and challenged with homologous and heterologous field antigens. Nine hundred and sixty chicks were vaccinated via coarse spray (d0). The treatments included (1) negative control (NC), (2) LCV and 0 g/ton PHY/PRO, (3) no LCV and 227g/ton PHY/PRO and (4) LCV and 227g/ton PHY/PRO. The trial design was a randomized complete block design with a factorial arrangement of treatments (60 birds/replicate). Significant differences among treatments means were determined using Tukey's-HSD test with a 5% level of probability. Standard performance parameters and coccidial lesion scoring were conducted 6 days post challenge. Two to four birds/replicate were challenged via gavage on 22, 28, and 35 d of age with the homologous antigen. On d 28 birds were also challenged with *E. maxima* and *E. tenella* (2 birds/replicate for each heterologous antigen). Challenged birds were housed separately from the other trial birds. For each challenge period, no significant interactions were detected therefore only main effects of treatments will be discussed. For the d 22 challenged birds, the average microscopic lesion score (MS) of chicks immunized with LCV was significantly

lower (0.46) compared to the MS from chicks that were not vaccinated (1.93). In addition, the average bird gain (ABG) from d 22-28 was significantly higher in the LCV treatment (432.6 g) compared to the ABG of the non-vaccinated treatment (378.8 g). When the birds were challenged with *E. tenella* at 28 d of age, the average MS of intestines from birds immunized with the LCV was lower ($P \leq 0.05$) compared to the MS of intestines from birds that were not vaccinated (0.53 vs 1.94). No differences in MS or ABG of *E. tenella* challenged birds were detected due to PHY/PRO supplementation. However, the ABG from d 28-34 from *E. tenella* challenged birds was higher in the LCV treatment (544.5 g) compared to ABG of birds not vaccinated (426.8 g). Similar results were found when birds were challenged with *E. maxima* on d 28. No differences in MS of birds challenged with homologous antigen on d 28 and 35 were detected. However the ABG from d 35-41 was improved ($P \leq 0.05$) in birds that received the 227 g PHY/PRO (662.6 g) compared to the ABG of 0 g PHY/PRO (552.5 g). It was concluded that the LCV was effective at reducing the negative effects observed in coccidiosis-challenged birds, and PHY/PRO product displayed efficacy in later challenged birds.

Key Words: broilers; coccidiosis; probiotics; phytogenics; live coccidia vaccine

347P Differential analysis of autophagy-related genes in broilers challenged with *Eimeria maxima* with or without *Clostridium perfringens*. Klao Runcharoon^{1*}, Nima Emami¹, Rami Dalloul¹, ¹University of Georgia, Athens, Georgia, United States.

Autophagy is a lysosomal degradative process that plays a central role in cellular differentiation, development, and homeostasis by removing defective organelles, protein aggregates, and intracellular microorganisms including parasites and bacteria. *Eimeria* species, including *E. maxima* (EM), are the major intestinal parasites in poultry and predisposing factors for necrotic enteritis (NE), an enteric disease caused by *Clostridium perfringens* (Cp) toxins. The objective of this study was to explore the effects of EM or EM+Cp infection on mRNA abundance of autophagy-related genes (ATGs). A total of 33 day (d)-old broiler chicks were individually wing-tagged and allocated to one of four treatment groups: non-challenged control (NC; n=4), EM only challenge (EM; n=9), EM + Cp #4 (Cp4; n=10), and EM + Cp #6 (Cp6; n=10). On d 14, birds were orally gavaged with 3,000 EM sporulated oocysts. Birds in the Cp4 and Cp6 groups were orally gavaged with approximately 1×10^8 CFU of either Cp #4 or Cp #6 on d 19 and d 20. On day 21, jejunal samples were collected to assess mRNA abundance of ATG. The data were analyzed using JMP (Pro16) and significance ($P \leq 0.05$) between treatments was determined by the LSD test. The results revealed that mRNA abundance of ATG4 and Rab7 (endosomal GTP-binding protein), ATGs related to the formation and elongation steps of the autophagy pathway,

was significantly greater in the Cp4 and Cp6 birds compared to those in NC and EM. mRNA abundance of Beclin-1 (involved in autophagosome formation) exhibited greater mRNA abundance in NC and Cp6 groups compared to EM and Cp4. mRNA abundance of ATG5 (associated with autophagosome formation) revealed greater mRNA abundance in NC, Cp4, and Cp6 groups compared to EM birds. mRNA abundance of ATG7 (an autophagy effector enzyme) was similar ($P > 0.05$) in all four groups. mRNA abundance of microtubule-associated protein B-light chain 3 (LC3B), involved in the elongation step, was significantly greater in EM birds compared to all other groups, while significantly lower in Cp4 and Cp6 birds than those of the NC group. NE appeared to affect the autophagy during elongation more than EM but did not correlate with NE lesion scores found only in Cp6 birds. However, LC3B, an ATG of mature autophagosomes, was highest ($P < 0.05$) in EM birds. These findings suggest that EM might have impacted the autophagy pathway by accumulating autophagosomes that were about to fuse with lysosomes at the time of sample collection. Collectively, autophagy is a dynamic process and potentially linked with both EM and NE challenges in broiler chickens warranting further mechanistic studies detailing the role of autophagy in the progression and prevention of these enteric diseases.

Key Words: broiler; necrotic enteritis; Autophagy; *Eimeria Maxima*; autophagosome

348P The prevalence of antimicrobial resistance genes in agreement with susceptibility patterns among extra-intestinal *Escherichia coli* isolated from layer chicken. Fozol Ovi^{1*}, Gregory Pharr¹, Martha Landinez¹, Reshma Ramachandran¹, Li Zhang¹, Anuraj Sukumaran¹, Pratima Adhikari¹, ¹Mississippi State University, Starkville, Mississippi, United States.

Avian pathogenic *Escherichia coli* (APEC) causes a wide range of diseases in poultry broadly termed as colibacillosis. One of the main challenges in controlling APEC is their unpredictable antibiotic resistance pattern which also poses a severe public health concern. Therefore, in this study we evaluated the prevalence of 11 antimicrobial resistance (AMR) genes in extra-intestinal *E. coli* and correlated them to phenotypic antibiotic susceptibility (PAS) towards 10 antibiotics. For this study, a total of 13 *E. coli* isolates were obtained from extra-intestinal tissues like heart and liver of layer chicken using MacConkey agar and confirmed by the presence of *ybbw* gene in a qPCR. Six of them were APEC isolates obtained from clinical case of colibacillosis and the remaining 7 were recovered from asymptomatic healthy layers. Each isolate was subjected to PCR test for 11 AMR genes; *blaTEM*, *tetB*, *tetA*, *qnr*, *aadA*, *aac3vIb*, *aaph(3)-Ia*, *sull*, *dfr7*, *dfr17* and *dfr1*. Additionally, PAS of the isolates were determined using Kirby-Bauer disk diffusion method. The data were analyzed in a binomial model using GLIMMIX procedure of SAS 9.4. The agreement between AMR genes and PAS was evaluated using Cohen's Kappa

test. For the binomial model $P < 0.05$ was considered significant. For Cohen's Kappa test, $P < 0.10$ was considered significant in order to retain moderate agreements ($k = 0.41-0.60$). In PCR test, all the AMR genes showed a similar prevalence between isolates of asymptomatic and colibacillosis infected hen ($P > 0.05$). AMR genes like *tetB* (30.77%), *tetA* (30.77%), *aadA* (38.46%), and *sulI* (46.15%) showed a high prevalence, whereas *blaTEM* (15.38%) and *aaph3* (15.38%) genes had lower prevalence. None of the isolates had *qnr*, *dfr1*, *dfr7*, and *dfr17* genes. Both, the colibacillosis and the healthy layer isolates showed a similar PAS towards all 10 antibiotics ($P > 0.05$). All 13 isolates were 100% susceptible to chloramphenicol, sulfamethoxazole, and ciprofloxacin. A higher degree of resistance was observed toward streptomycin (30.77%), gentamicin (30.77%), and tetracycline (38.46%). Cohen's Kappa test revealed that the PAS to tetracycline is congruent with the presence of *tetB* and *tetA* genes ($P = 0.07$). Gentamicin PAS corresponded to the occurrence of *aadA* gene ($P = 0.07$), and streptomycin PAS corresponded to both *aadA* ($P = 0.07$) and *aaph(3)-Ia* ($P = 0.02$) genes. These results indicate, antibiotic susceptibility and AMR gene profile of APEC isolates are comparable to that of healthy layer isolates. AMR genes *tetB*, *tetA*, *aadA* and *aaph(3)-Ia* in agreement with PAS of tetracycline and gentamicin. Prohibition of chloramphenicol, sulfamethoxazole, and ciprofloxacin in poultry effectively increased the susceptibility of avian *E. coli* to those drugs.

Key Words: APEC; Colibacillosis ; AMR profile; Phenotypic antibiotic susceptibility; Anti-microbial resistance profile

349P Complete genome sequences of six multidrug-resistant avian pathogenic *Escherichia coli* strains isolated from broilers exhibiting colibacillosis. Linan Jia^{1*}, Priyanka Devkota¹, Mark Arick II¹, Chuan-Yu Hsu¹, Daniel Peterson¹, Jeffrey Evans², Anuraj Sukumaran¹, Li Zhang¹, ¹Mississippi State University, Mississippi State, Mississippi, United States, ²USDA, Mississippi State, Mississippi, United States.

Colibacillosis is a disease that causes significant economic losses in the poultry industry. Avian pathogenic *Escherichia coli* (APEC) is the main causative agent of this disease. Therefore, there is a critical need to identify the antimicrobial resistance properties of APEC in commercial poultry farms to develop efficient preventive measures to control the outbreak of colibacillosis. The goal of this work was to characterize the antimicrobial resistance properties of APEC by sequencing the genomes of six antibiotic-resistant *Escherichia coli* strains isolated from broilers exhibiting colibacillosis. In our previous study, six *E. coli* strains were isolated from heart, air sac, lung, and blood samples of broilers exhibiting colibacillosis infections. In this study, we sequenced and annotated each individual complete genome from these six *E. coli* isolates

to identify the profiles of the antimicrobial resistance genes (ARGs). To get the genome with complete and accurate sequences, whole-genome sequencing was performed using the Nanopore long-read sequencing (Nanopore GridION) coupled with the Illumina short-read sequencing (Illumina HiSeq X-Ten). The genome sequences were then assembled in Canu v.1.9 using the Nanopore reads and polished in Pilon v.1.23 using the Illumina reads mapped with Burrows-Wheeler Aligner (BWA) v.0.7.17-r1188. The assembled genome sequences were annotated using the Prokaryotic Genome Annotation Pipeline (PGAP) from NCBI. Multilocus sequence typing (MLST), phylogenetic typing, and serotyping (O-antigen and flagellin genes) were performed for further classification. ARGs were determined using ResFinder v.4.1. The chromosome sizes of the genome assemblies ranged from 4,724,195 to 5,174,435 bp and their plasmid sizes ranged from 34,981 to 203,860 bp. A total of 17 ARGs were detected, giving resistance to antibiotics belonging to different classes or groups, including peroxides, aminoglycosides, folate pathway antagonists, macrolides, fluoroquinolones, quaternary ammonium compounds, aminocyclitols, tetracyclines, and beta-lactams. Five distinct sequence types (STs) were identified, including ST69 and ST117 which are linked to human extraintestinal illnesses. The findings of this study showed that ARGs were widely distributed in *E. coli* isolated from colibacillosis-infected chicken. The genome sequences will help the research on the resistance characteristics and pathogenetic pathways of *E. coli* in poultry farms.

Key Words: colibacillosis; whole-genome sequencing; antimicrobial resistance genes; Avian pathogenic *Escherichia coli*; poultry farm

350P In silico prediction of vaccine candidates against colibacillosis in poultry using reverse vaccinology. Linan Jia^{1*}, Sabin Poudel¹, Priyanka Devkota¹, Mark Arick II¹, Chuan-Yu Hsu¹, Daniel Peterson¹, Jeffrey Evans², Li Zhang¹, ¹Mississippi State University, Mississippi State, Mississippi, United States, ²USDA, Mississippi State, Mississippi, United States.

Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis, and an effective vaccine against APEC infection is needed for preventing this devastating disease. Vaccines designed to protect against specific hypervirulent APEC strains lack efficacy against genetically diverse strains circulating in different poultry flocks. Thus, there is a need to identify APEC core genes and conserved antigens for the development of broadly protective vaccines. The current study aimed to identify universal antigens present in APEC strains, which could be utilized in vaccine development to reduce poultry APEC infection at a larger scale on the farm. The whole-genome sequences from pathogenic isolates were used in initial vaccine candidate identification via reverse vaccinology strategy in this study. Whole-genome sequences of 25 APEC isolates from poultry

exhibiting colibacillosis were used. Core-genome was predicted and annotated using the Roary pan-genome pipeline. The subcellular localization, transmembrane helix number, and adhesion probability were predicted using PSORTb, TMHMM, and SPAAN pipelines, respectively. The extracellular and outer membrane proteins with 0 or 1 transmembrane helix and with an adhesion probability ≥ 0.5 were selected for further antigenicity screening using VaxiJen. The conservation within pathogens and uniqueness against the host of selected proteins were confirmed by tblastn using the NCBI *E. coli* database and BLASTp using the NCBI *Gallus gallus* database, respectively. The immune response for all protein candidates was predicted using BCPreds for the B-cell epitope density count and using the Immune Epitopes Database (IEDB) for immunogenicity (with a score ≥ 0) prediction to select the final vaccine candidates. A total of 3,061 core-genome protein-coding sequences from 25 APEC isolates were predicted, including 44 proteins that met the criteria of subcellular localization, transmembrane helix number, and adhesion probability. Among them, 32 proteins were antigenic (antigen score ≥ 0.5) and highly conserved across the *E. coli* database. Finally, one protein related to nickel and zinc acquisition (metal-pseudopaline receptor CntO-like), three proteins related to adhesion (basal-body rod modification protein, flagellar L-ring protein, and outer membrane protein X), five proteins related to the membrane and cell wall function (malto porin, mltA-interacting protein, outer membrane porin N, nucleoside-specific channel-forming protein, and putative acyl-CoA thioester hydrolase), two proteins related to toxin production (lipid A palmitoyltransferase and phospholipase A1), and two proteins with unknown names and functions were predicted as potential vaccine candidates.

Key Words: avian pathogenic *Escherichia coli*; Colibacillosis ; reverse vaccinology; vaccine candidate; in silico prediction

351P Endectocides: For the simultaneous control of multiple vector-borne diseases in poultry. Koyle Knap^{1*}, Cassandra Durden¹, Macie Garza¹, John Carey¹, Sarah Hamer¹, Gabriel Hamer¹, ¹*Texas A&M University, College Station, Texas, United States.*

Zoonotic pathogens continue to emerge and threaten both human and animal health. Increased urbanization, globalization, and climate change have facilitated increased transmission of numerous infectious agents, especially those vectored by arthropods. The most common form of arthropod vector control is environmental insecticides. In many cases the use of environmental insecticides are limited, ineffective, and harmful to the environment. An emerging approach is the use of host targeted insecticides (endectocides). It has been shown that domestic poultry provide bloodmeals to triatome vectors of the Chagas disease parasite. Our hypothesis is that arthropod vectors that feed on endectocide-treated poultry will have a

higher rate of mortality compared to control groups. This trial evaluated the efficacy of three endectocide products: Safe-Guard® Aquasol (Fenbendazole), Ivomec® Pour-On (Ivermectin), and Bravecto® (Fluralaner), dosed orally. Seventy laying hens were obtained from a commercial source. Hens were housed in an environmentally controlled layer house in individual cages with a nipple waterer serving individual hens. Safe-Guard® Aquasol (Fenbendazole) and Ivomec® Pour-On (Ivermectin) treatments were added to a gravity flow nipple watering system that only delivers medicated water to the respective treatment group for five days as recommended by the manufacturers. A weighed, dyed (for visualization) piece of Bravecto® (Fluralaner) was given to hens mixed with their feed based on weight. Three starved *Triatoma gerstaeckeri* (Kissing Bug) were allowed to feed for forty-five minutes on live, restrained hens at day three, day seven and day fourteen post-treatment while monitoring the fed vectors for survivability. Two hens were used for each treatment group. Statistical analysis showed that mortality of kissing bugs feeding on Bravecto® (Fluralaner) treated hens was significantly higher (<0.001) compared to hens treated with Safe-Guard® Aquasol (Fenbendazole), Ivomec® Pour-On (Ivermectin) and control hens at all three time points. There were no significant differences found for vector mortality between Safe-Guard® Aquasol (Fenbendazole), Ivomec® Pour-On (Ivermectin) and control treated hens at the chosen time points. In conclusion we have shown that Bravecto® (Fluralaner) administered to layer hens can suppress populations of kissing bugs.

Key Words: Poultry ; Insecticide; Endectocide; Triatome; Mosquito

352P Local and systemic inflammatory and antibody responses to intradermal administration of killed *Salmonella* vaccine in different vaccine vehicles. Jossie Santamaria^{1*}, Chrysta Beck¹, Marites Sales¹, Gisela Erfl¹, ¹*University of Arkansas System, Division of Agriculture, Fayetteville, Arkansas, United States.*

The choice of vaccine vehicles may affect vaccine efficacy. Information on the immune effects of vehicles for *Salmonella* bacterin vaccination is limited. The objective of this study was to determine the local tissue and systemic inflammatory responses, and the antibody production to a first injection of formalin killed *Salmonella* Enteritidis (SE) vaccine in three vehicles (i.e., oil-water emulsion (Oil), 15% alum adjuvant (Alum), or endotoxin-free PBS). Using the pulp of growing feathers (GF) as the test-tissue, GF-pulps of 14-wk-old Light-brown Leghorn pullets were intradermally injected with either SE-Oil, SE-Alum, SE-PBS, or vehicles alone; 10 μ L/GF, 18 GF/bird, 4 birds/treatment. Injected GF and heparinized blood samples were collected before (0h) and at 6, 24, 48, and 72h post-GF injection for leukocyte population analyses. Blood collected at 0, 3, 5, 7, 10, 14, 21, and 28d post-GF injection was used to determine plasma levels of SE-specific IgG, IgM, and

IgA by ELISA. Effects of time and treatments on tissue and blood leukocyte profiles were analyzed by two-way ANOVA. Leukocyte-infiltration (% pulp cells), consisting primarily of heterophils and monocytes, was highest at 6h and gradually declined to above pre-injection levels by 72h ($P<0.001$). Heterophil levels were highest with SE-Alum, intermediate with SE-PBS, and lowest for SE-Oil ($P<0.001$). SE-Oil recruited the highest levels of macrophages ($P<0.001$). While few lymphocytes were recruited, total lymphocyte levels were highest with SE-Alum and SE-PBS compared to SE-Oil ($P=0.03$). The local inflammatory activity was reflected in changes among WBC populations, with a 3-4-fold increase ($P<0.001$) in % heterophils at 6h, no change in % monocytes, and a drop in % lymphocytes at 6h. Blood heterophil proportions remained elevated at 24h ($P<0.001$), whereas lymphocyte levels returned to pre-injection levels by 24h. The 6-h drop in % lymphocytes was due to T cells. SE-formulations did not affect the proportions among WBC. Two-way repeated measures ANOVA of the SE-specific IgM, IgG, and IgA antibody responses revealed only time effects ($P<0.001$) and no treatment differences. IgM levels were maximal at 5d, remained at this level until 7d, before gradually declining. IgG reached maximal levels by 7d and 10d, and remained near peak levels thereafter. IgA increased to the highest levels by 10d, and then gradually declined, remaining above pre-injection levels by 28d. Although few differences in the quantitative and qualitative aspects of the immune responses to the SE-formulations were observed, the simultaneous analysis of inflammatory and antibody responses is a comprehensive approach to study the contributions of vehicles in *Salmonella* bacterin vaccines.

Key Words: Inflammatory response ; Antibody response ; Light-brown Leghorn pullets; *Salmonella* bacterin vaccine ; Vaccine vehicles

353P A post-mortem case study: Gross pathological /necropsy investigation of internal laying in Peahens. Asad Khan^{1*}, Ahmad Din Anjum¹, ¹Riphah College of Veterinary Sciences, Lahore, Pakistan, ²UVAS, Lahore, Pakistan.

This study reports the gross pathological and necropsy investigation of internal laying in peahens. Birds with fully formed egg in peritoneal cavity is called ectopic egger or internal laying and this occurs due to anti peristaltic movement in oviduct. It reduces the productive efficiency of birds and results in the death of birds. One dead adult 6 year's old Indian pea hen (*Pavo cristatus*) was brought for necropsy examination at the Department of Pathology, Riphah College of Veterinary Sciences (RCVetS), Lahore, Pakistan. Histology revealed that bird was anorexic and lethargic from last 48 hours and died suddenly. The bird was reported belong to a confined flock of 30 peafowls. Diarrhea and ataxia were few clinical signs noted before death. Upon external examination, body condition of bird was found poor, dehydrated with sunken eyes, vent gleet and pasting

around vent. During necropsy examination it was found that oviduct was blocked between magnum and isthmus part due to the presence of a fully calcified egg (weight about 96 grams and 3.2 inches in length). Ovaries were normal with full ovarian clutch. Left unilateral nephritis was also observed. Presence of internal egg has been commonly reported in commercial layers because of inbreeding and can be reduced to promote cross breeding but it was not yet reported in peahens.

Key Words: Internal laying; Peahen; Peritoneal cavity

354P Egg production and quality in 30-week-old Lohmann LSL lite hens fed various doses of enzymatically treated yeast supplement. Colin DeCloeet^{1*}, Anderson Maina¹, Hagen Schulze², Elijah Kiarie¹, ¹University of Guelph, Guelph, Ontario, Canada, ²Livalt, Peterborough, United Kingdom.

High-density egg production systems present hens with performance challenges, stress, and disease susceptibility. The role of functional feedstuffs such as enzymatically treated yeast (HY40; 40% yeast cell wall components) on aspects of egg production and quality is unexplored. We assessed the effects of supplementing HY40 in layer diets on hen-day egg production (HDEP), feed intake (FI), feed conversion ratio (FCR), and egg quality attributes. A total of 160 30-wk-old Lohmann LSL lite hens were placed in 40 enriched cages (four birds/cage) based on body weight (BW) and distributed to 5 diets in a completely randomized design for an eight-week trial. The diets were corn-soybean meal-based with 0.0, 0.025, 0.05, 0.1 or 0.2%HY40. Feed and water were provided freely; HDEP and FI were monitored weekly, whereas egg components, eggshell breaking strength (ESBS), and thickness (EST) were monitored biweekly. With the cage as the experimental unit, data were subjected to GLM procedures of SAS with diet, sampling time (ST), and associated interactions as fixed factors. LSmeans for diet were separated with dose-response curve and for ST with Tukey. There were no diet and ST interactions ($P>0.05$) on response criteria. There was a linear ($P=0.039$) and quadratic ($P=0.024$) increase in FI response to HY40. There was no ($P\geq 0.109$) diet effect on HDEP; the HDEP was 98, 99, 96, 96 and 95%, respectively, for 0.0, 0.025, 0.05, 0.1 or 0.2%HY40. Supplemental HY40 increased EW and egg mass (HDEP*EW) linearly ($P\leq 0.01$) and quadratically ($P\leq 0.02$). Specifically, the egg mass was 57.6, 61.2, 59.4, 58.8 and 59.1 g/bird/d, respectively for 0.0, 0.025, 0.05, 0.1 or 0.2%HY40. Albumen increased linearly ($P=0.03$), and egg yolk tended to decrease linearly ($P=0.08$) in response to HY40. There were no ($P>0.05$) diet effects on FCR, ESBS, EST, and eggshell. There were no ($P>0.05$) age effects on HDEP and eggshell attributes, but birds ate more feed as they aged. However, FI, FCR, and egg yolk increased ($P<0.05$), and albumen decreased with age. In conclusion, FI increased due to diet and aging factors. Although HY40 had no effects on the rate of egg production and FCR, birds fed HY40 laid heavier eggs with a larger

proportion of albumen, suggesting improved amino acid assimilation.

Key Words: feed intake; egg quality; egg weight; enzymatically treated yeast ; Hen-day egg production

355P Litter moisture and depth variations in the cage-free houses during pullet production. Xiao Yang^{1*}, Lilong Chai¹, Ramesh Bist¹, Sachin Subedi¹, ¹*University of Georgia, Athens, Georgia, United States.*

Litter quality is critical to birds' health and welfare in cage-free laying hen houses. Laying hens have up to 70-80 weeks of age for each laying cycle on commercial farms. Therefore, the early stage of litter quality matters to indoor air quality and birds' footpad health in the whole production cycle. The primary objective of this study was to evaluate litter quality, and its variability and influential factors in four research cage-free layer facilities (denoted as room A, B, C, and D, each had floor area of 18.6 m² with 200 Hy-Line W-36 pullets) on the Poultry Research Farm at the University of Georgia in Athens, GA. In this study, pine shavings were used as bedding materials (initial depth was 2.5 cm). Air temperature and relative humidity were monitored continuously with HOBO MX2300 Data Loggers. Litter samples were collected from four representative zones (denoted Z1 –feeders' zone, Z2 – close to walking zone, Z3 –nipple drinkers' zone; and Z4 – perching zone). Litter quality such as litter depth, litter density, mass production, and moisture content (LMC) were measured weekly. Litter density was estimated by using a 500 mL measure cup and a digital scale; moisture content was measured by drying the litter samples in a 105 °C oven for 24 h; litter generation was estimated based on weekly litter depth, density, and floor area of each room. To quantify the difference in litter quality between rooms and zones in each room over time, one-way ANOVA and Tukey HSD analysis were performed using JMP software. The difference was considered significant at $p < 0.05$. There are significant differences in LMC, depth, density, and generation in four rooms between week 1 and week 28 ($p < 0.05$) (e.g., LMC was increased from $8.6 \pm 0.2\%$ to $11.9 \pm 0.9\%$ by from week 1 to week 28. Meanwhile, the litter depth was increased from 2.5 cm to 4.3 cm. For different zones in the same room, the LMC, litter depth, density, and litter generation were observed different ($p < 0.05$). The highest LMC and litter depth were observed in Z3 (i.e., 11.4% LMC and 5.8 cm depth). There was no apparent change in litter density across zones (range = $0.36\text{--}4\text{ g cm}^{-3}$). Findings of this study provides reference for commercial cage-free farms to monitor and manage litter quality for a better animal wellbeing.

Key Words: animal welfare; litter moisture; Cage-free production; litter depth

356P Laser environmental enrichment yields greater breast muscle quality in Ross 708 broilers without impacting growth performance. Meaghan Meyer^{1*}, Anna

Johnson¹, Elizabeth Bobeck¹, ¹*Iowa State University, Ames, Iowa, United States.*

Genetic selection for feed efficient birds with high breast yields and fewer days to market has inadvertent effects on broiler behavior, welfare, and meat quality. Woody breast and white striping are significant *pectoralis major* myopathies prevalent in fast-growing commercial broilers. The effects of voluntary broiler exercise stimulated by the environment on these disorders have not been clearly established. A second-generation laser enrichment device previously shown to induce activity in both Ross 308 and 708 birds was implemented to stimulate exercise. Therefore, the experimental objectives were to measure effects of the updated laser device on broiler performance and breast muscle quality. 1360 Ross 708 broilers (680 birds per treatment, laser-enriched or control; 34 birds/pen) were randomly assigned to pens raised for 49d. Laser-enriched birds were exposed to six-minute laser periods (four laser dots per device, one device per two laser-enriched pens) four times daily for the study duration. Growth performance outcomes were calculated by pen and averaged per bird by performance period. A subset of 70 broilers ($n=35$ laser-enriched and 35 control) were sampled for breast muscle width, breast fillet height and weight, and fillet woody breast (WB) and white striping (WS) score at d42 and d49. Following both sampling timepoints, compression force (one day post-mortem) and water-holding capacity (two days post-mortem) were analyzed. Performance and breast muscle data were analyzed using Proc Mixed in SAS Version 9.4 (Cary, N.C.) with the fixed effect of enrichment. Percent distribution of WB and WS scores were analyzed using a Chi square likelihood ratio. Results showed that feed intake, weight gain, FCR, and body weight were not affected by laser enrichment ($P > 0.05$). However, laser exposure increased breast width and fillet weight at d42 by 1.08cm (width of entire breast; $P < 0.05$) and 30g (left fillet weight; $P < 0.05$). At d49, breast fillet height was increased in laser-enriched birds (0.42cm increase; $P < 0.05$). At d42, laser enrichment had an impact on WS score ($P < 0.05$), with a 24% reduction in severe WS, an improvement that was also observed on d49, where there was a 15% reduction in severe WS ($P < 0.10$). Percent distribution of WB score was numerically improved due to laser enrichment, with 11% less severe WB observed on d42 and 18% less severe WB observed in laser-enriched broiler breasts on d49. Mechanical compression force of raw fillets was not affected by enrichment ($P > 0.05$), but water-holding capacity was improved in d49 laser-enriched broiler breast fillets ($P < 0.01$). Overall, a laser-enriched environment benefitted breast muscle quality, likely due to increased activity in laser-enriched birds.

Key Words: broiler; meat quality; white striping; woody breast; environmental enrichment

357P Pulsed Alternating Wavelength System (PAWS) lighting in layer house reduces brain serotonin turnover

rate suggested improved welfare. Sara Tonissen^{1*}, Brittney Emmert¹, Meagan Abraham¹, Darrin Karcher¹, Gregory Fraley¹, ¹Purdue University, Olmsted Falls, Ohio, United States.

Lighting can have profound effects on the health, production, and welfare of all poultry species, and the layer chicken is no exception. Like all poultry species, chickens are seasonal breeders and thus require artificial light to maintain long daylengths for maximum egg production. However, there are no standards for lighting systems in commercial layer houses. Very few studies have evaluated the growth and welfare of layers housed under different lighting systems. A novel LED technology has recently been introduced referred to as Pulsed Alternating Wavelength System (PAWS) that delivers multiple wavelengths of light to animals. Previous studies from our labs have suggested that PAWS may improve wellbeing of other poultry species. Decades of research have shown that reduced brain serotonin turnover rates are related to improved wellbeing and welfare of many vertebrate species, including poultry. The goal of this study was to determine the effects of PAWS on brain serotonin turnover rates. We tested the hypothesis that PAWS lighting would decrease brain serotonin turnover rates compared to control lighting. To test this hypothesis, brains were collected from layer hens leading up to peak production (22-32 weeks) and again at a year of age (weeks 50-52) that were housed under PAWS or control lighting (fluorescent white light) (N = 10 per week, per lighting type). These brain samples were immediately frozen on dry ice and stored at -80° C until they were microdissected into dorsal and caudal raphe, diencephalon, and telencephalon samples. Samples were processed and assayed using mass spectrometry for serotonin and its metabolites. Data were analyzed using ANOVA by age and lighting treatment with a Fisher's PLSD *post hoc* test. Compared to controls, serotonin turnover rates were reduced in all 4 brain areas in the peak production PAWS birds ($p < 0.01$). Serotonin turnover rates were reduced in all groups at the older ages ($p < 0.001$), as is well established for vertebrates. PAWS lights showed a slight, ($p < 0.05$), increased serotonin turnover at 50 weeks but a slight ($p < 0.05$) decrease in serotonin turnover rates at 52 weeks compared to controls. These results suggest that lighting systems can be used to alter brain serotonin activity, and that PAWS lighting may improve layer hen wellbeing as has been suggested in other poultry species.

Key Words: laying hen; light; wellbeing; 5-HIAA; homovanillic acid

358P Not presented.

359P Everyday feeding: an alternative to broiler breeder pullet skip-a-day feeding program and its implications on growth, gastrointestinal and skeletal characteristics. Luis Avila^{1*}, Kelly Sweeney¹, Camille Evans¹, Dima White¹, Woo Kim¹, Prafulla Regmi¹, Jeanna

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Given the continuous selection of broiler lines for efficient feed conversion and meat yield, broiler breeders are feed restricted to control BW gain to promote reproduction. Skip-a-day (SAD) involves feeding birds every other day and feeding twice as much on feed days. It is uncertain if SAD is favorable for the modern breeder pullet physiological development and subsequent reproduction compared to alternative methods like every day (ED) feeding. The objectives of this study were to compare ED and SAD programs and their impact on pullet growth, intestinal and skeletal characteristics during rearing. At d 0, Ross 708 (Aviagen) pullet chicks ($n = 1,778$) were randomly assigned to 7 floor pens ($n = 254$ per pen) and full-fed a common crumbled starter diet through wk 3. Three pens were fed using the ED and 4 pens with the SAD program through wk 21 using chain feeder systems. Both feeding methods provided the same feed amount per bird over a two-day period, and feed allowance was equally adjusted every wk. A 2-stage grower diet program was fed after wk 3. ED and SAD grower diets were formulated to be iso-caloric and iso-nitrogenous, with the only difference that ED diets had 15 % more crude fiber from whole oats and soy hulls to increase volume. All pullets were weighed at 5, 10, 15, and 20 wk of age. Intestine samples were obtained from pullets at wk 4, 10, 16, and 22 and processed for histological analysis. Pullets were scanned using dual energy X-ray absorptiometry (DEXA) to obtain body bone density and proximal composition. Data were analyzed using a GLM procedure and LSMEANS for mean separation with SAS v 9.4 at a significance of $P \leq 0.05$. ED-birds were heavier at wk 10, 15, and 20 by 4.5 %, 3.5 % and 4.3 %, respectively compared to SAD pullets ($P < 0.001$). In contrast, pullet BW uniformity was unaffected by feeding methods through wk 20 ($P \geq 0.443$). Feeding method did not affect pullet mortality ($P \geq 0.283$), although ED program improved feed conversion (6.85 vs. 7.00 kg per live pullet through 21 wk; $P = 0.001$). At most sampling ages, SAD-birds had heavier crops, proventriculus, and livers compared to ED pullets ($P \leq 0.036$). Duodenal, jejunal and ileal villi goblet cells were more abundant in pullets reared under the ED at wk 4 and 10 ($P \leq 0.050$). Scan results show that ED-pullets had improved bone mineral density at wk 7, 15, and 19 ($P \leq 0.026$), and higher bone mineral contents at wk 7 ($P = 0.012$). In conclusion, the ED feeding program did not negatively impact pullet BW, improved feed utilization, altered intestinal characteristics, and improved whole body bone density of pullets. Future research will focus on subsequent reproductive performance of hens previously reared under these two programs.

Key Words: bone quality; broiler breeder pullet; gastrointestinal tract; feed restriction; rearing

360P Effects of endosperm hardness and drying temperature on pellet durability index of broiler diets.

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Corn kernel hardness and drying temperature affect particle size and its distribution post-grinding, starch properties, non-starch polysaccharide composition, vitreousness, and consequently may affect pellet production rate and pellet durability index (PDI). The objective of this study was to evaluate the effects of corn endosperm kernel hardness (KH) and the drying temperature (DT) post-harvest on the feed pelleting process and PDI of broiler diets. Two corn varieties with average and hard KH were planted and harvested simultaneously under similar agronomic conditions, and dried in an industrial forced-air dryer at 35 or 120 °C. The corn vitreousness post-drying was determined for average KH-35°C (59.83%), average 120°C (61.36%), Hard KH 35°C (62.05%), and Hard 120°C (63.60%). Corn was cleaned in a seed-cleaner and ground in a hammermill with settings targeting particle sizes between 700 and 800 µm for grower and finisher broiler diets. A basal diet was produced, and only corn varied according to the treatment. The grower diets had 57.5% corn, 32.0% soybean meal, and 4.5% added poultry fat, while finisher diets included 61.2% corn, 27.8% soybean meal, and 5.6% poultry fat. All meals were steam conditioned at 83 to 85°C for 30 seconds. The hot pellet temperature was determined to be between 83.9 and 85.7 °C. Five pellet samples per mixed treatment were evaluated for PDI using a Holmen tester for 30 seconds. Four treatments were evaluated, resulting from a 2x2 factorial arrangement in a completely randomized design and means were separated using the Student's t-test and Tukey's HSD. An interaction effect ($P<0.05$) was only observed in the production rate of the finisher diets. In corn dried at 35 °C, diets containing average KH corn had a lower production rate (15.8 kg/min) than diets with hard KH (16.7 kg/min). Higher production rates were observed ($P<0.05$) when diets contained the hard KH corn than when diets had the average KH. The temperature variation between conditioner and hot pellets was affected ($P<0.01$) in both diets by KH and only in the finisher diets by DT. Only in grower diets, corn dried at 120°C had a lower ($P<0.05$) production rate (14.8 kg/min) than diets with corn dried at 35°C (15.1 kg/min). No interaction effects were observed ($P>0.05$) on PDI. Corn KH ($P<0.01$) and DT ($P<0.001$) affected grower PDI. Better pellets were observed with hard KH corn and corn dried at 35°C. Only DT effects ($P<0.05$) were detected in the finisher diets, and higher PDI was observed in diets with corn dried at 120°C. In conclusion, diets containing the hard KH corn had a better production rate and pellet durability, but drying temperature post-harvest can cause variability in these parameters in broiler diets.

Key Words: drying temperature; pellet durability index (PDI); Corn kernel hardness; production rate

361P Endosperm kernel hardness and moisture at harvest affect corn particle size and its distribution post roller milling. Jhon Mejia-Abaunza^{1*}, Paula Lozano-Cruz¹, Joaquin Cabanas-Ojeda¹, V.L. Aragao- Neto¹, Adam Fahrenholz¹, Samuel Brown¹, Edgar Oviedo-Rondón¹, ¹*North Carolina State University, Raleigh, North Carolina, United States.*

Endosperm kernel hardness causes variation in particle size after grinding and can influence nutrient digestibility, poultry gastrointestinal development, and performance. Moisture (MO) at harvest may modify this response, but data quantifying this effect is scarce. This study determined the impact of corn variety and MO at harvest on the geometric mean diameter (D_{gw}) and geometric standard deviation (S_{gw}) by weight after roller milling. Four yellow dent corn varieties DK 6869, 6205, 6357, and 6208 were planted under similar agronomic conditions and harvested with 34, 28, 22, and 16% MO. All corn kernels were mechanically shelled and dried in an air-forced oven at 120 °C. The drying time varied by initial MO between 3 h and 15 min to obtain corn MO near 13%. Vitreousness and MO were measured. All corn treatments were sieved and cleaned before grinding in a roller mill with a 25-25 gap between the rolls. This roller gap was selected by targeting a D_{gw} close to 1,050 µm for feeding laying hens. Five samples per corn treatment and grinding were analyzed by the sieving method ASAE S319.3. Data was analyzed in a 4x4 factorial arrangement in a completely randomized block design, and means were separated with Tukey's test. Final MO post drying varied ($P<0.001$) between 9.3 and 12.8%, but no effect of MO pre-grinding was detected ($P>0.05$) on D_{gw} or S_{gw} . The average vitreousness of these corn varieties post-drying was 59.46, 60.10, 57.64, and 61.06%, respectively. Vitreousness varied by variety ($P<0.001$) and increased linearly ($P<0.001$) as MO at harvest decreased. Interaction effects ($P<0.001$) were observed in D_{gw} . The D_{gw} increased linearly as corn MO at harvest decreased ($P<0.001$), but the effect depended on corn variety. Variety DK6208 had the lowest D_{gw} (842 µm) with 34% MO at harvest. DK6208 had the lowest D_{gw} at all MO levels (866 µm). In contrast, DK6205 had the largest D_{gw} (1,066 µm) with 16 or 28% MO at harvest, which was similar to DK6869 with 16% (1,063 µm) or DK6357 with 22% (1,048 µm) MO. No interaction effect was observed ($P>0.05$) on S_{gw} . As corn MO at harvest decreased, the S_{gw} ($P<0.001$) was reduced from 4.87 to 4.20 mm. Corn varieties differed ($P<0.001$) in S_{gw} . The DK6208 had the highest S_{gw} (4.81 mm), while DK6869 had the lowest (4.37 mm), and the others were intermediate. Neither D_{gw} nor S_{gw} were linearly correlated ($P>0.05$) with vitreousness, but they were correlated ($P<0.01$) with MO pre-grinding, $r = 0.58$ and -0.31 , respectively. In conclusion, corn variety and MO level at harvest affected D_{gw} and S_{gw} in a roller mill. Some corn varieties produce smaller and more variable particle size than others. Controlling these factors could minimize variability in feed quality and poultry production.

Key Words: particle size; roller mill; moisture ; vitreousness; Corn variety

362P Evaluating bed bug trap and lure performance for monitoring in poultry facilities. Maxwell Pasquinelli^{1*}, Kayla Durning¹, Nuket Acar¹, Nina Jenkins¹, Paul Patterson¹, ¹*Pennsylvania State University, University Park, Pennsylvania, United States.*

Bed bug (BB) infestation is challenging for bird welfare, care-taker comfort and the cost to rid poultry facilities and homes of this pest. IPM for fly and rodent early detection often begins with indexing their numbers and location to arrive at thresholds of infestation. Commercial traps are marketed to monitor BB infestations in residential settings. However, monitoring in poultry facilities requires traps to work with thousands of poultry hosts, elevated CO₂ and large quantities of dust and dander. Our objective was to evaluate commercial traps with (+) and without (-) their lures for application with commercial poultry. We evaluated 3 traps: the SenSci Pyramid, Nattaro and Delta-5 wifi each +/- lures. Arenas (95 x 95 x 7cm) covered with craft paper were used to study the impact of treatments on final bed bug location within the arena. Harlan BB were isolated from a colony jar, then given a blood meal. Groups of 10 adult males were placed in 50ml cups with filter paper as harborage. The tops were covered with fine mesh and held in an 21C incubator prior to use. Two traps of the same treatment (+/- lure) were placed on opposite sides of each arena. For each replicate (n=12) a cup of BB was opened and inverted in the center of the arena at 9am and allowed to acclimate for 8h. At 5pm the cup was removed, and BB were left undisturbed until 5pm the next day. BB number and location was recorded: A. remained on filter paper B. loose in arena, C. inside trap + lure, D. outside trap + lure, E. inside trap – lure, F. outside trap – lure. Data were analyzed by two-factor ANOVA using PROC GLM procedures of SAS Institute Inc. with the main effects of BB trap and arena on BB locations. Significance was set at ($P<0.05$). Contrast analysis was used to determine if traps +/- lures were significant. Results indicated for BB on traps the Delta-5 captured more BB, at a higher percentage than the Pyramid when no lure was deployed ($P=0.087$ and 0.056 , respectively) while the Nattaro was intermediate and not different from the other treatments. There was also a significant trap x arena interaction ($P<0.014$). When BB location on or in a trap was combined, the trend when no lures were deployed was again for the Delta-5 to attract more BB, at a higher percentage than the Pyramid with the Nattaro intermediate ($P=0.065$ and 0.042 respectively). Comparing lures vs. no lures, the BB/trap (2.58 vs. 1.61) and percentages (7.2 vs. 4.5%) suggest traps with lures attract more, however $P=0.099$. Finally, BB in traps averaged only 0.29 /trap compared with 4.33 remaining on the filter paper. Despite this result, the traps/lures herein have potential for BB monitoring, however, further testing in commercial poultry houses is recommended.

Key Words: poultry; integrated pest management; bed bugs; traps; lures

363P Decreasing contact dermatitis with circulation fans in commercial broiler houses. Zoie McMillian¹, Jonathan Moyle¹, Shawna Weimer^{1*2}, ¹*University of Maryland, College Park, Maryland, United States,* ²*University of Arkansas, Fayetteville, Arkansas, United States.*

Footpad dermatitis (FPD) and hock burn (HB) are forms of contact dermatitis on the skin of the ventral footpads and caudal (back) hock joint of broilers, respectively. These conditions, which can cause painful lesions if severe, are a threat to broiler health and welfare. Ventilation is an important management factor in broiler production systems; additional air movement in commercial houses could positively affect the welfare of broilers by reducing FPD and HB prevalence. The objective of this study was to evaluate the effect of circulation fans on the FPD and HB prevalence of broilers in commercial organic houses and it was hypothesized that broilers raised in the houses with circulation fans would have less FPD and HB than broilers raised in the houses without circulation fans. This study was conducted in 4 commercial organic broiler houses on a farm in eastern Maryland in October and November 2021. Two, 60×600 ft. houses had 16 high-capacity circulation fans (fan treatment) and two houses did not (control). House was the experimental unit ($N=4$ houses). Each house was divided into 4 equal quadrants for welfare assessments at 3 and 6 weeks of age. During the welfare assessments in each house, 20 birds within each quadrant (80 birds/house) were scored for FPD and HB on a 0 to 2 scale where 0 indicated no evidence of dermatitis, 1 indicated moderate evidence of dermatitis, and 2 indicated severe dermatitis. Right and left scores were averaged for analysis. The effect of the fan treatment or control on FPD and HB score data was analyzed with chi-square tests. At week 3, prevalence was low (FPD was 5% and HB was 0%) and there was no treatment effect. Both FPD and HB prevalence increased with bird age ($P<0.0001$). At week 6, a greater prevalence of FPD (79% vs. 61%) ($P=0.0002$) and HB was observed in control houses than in the treatment houses (57% vs. 28%) ($P<0.0001$). The results of this study suggest that the addition of circulation fans in commercial broiler houses could aid in decreasing FPD and HB prevalence. Thus, high-capacity circulation fans could potentially be a cost-effective way for producers to increase broiler welfare.

Key Words: Broilers; Welfare ; Footpad Dermatitis; Hock Burn; Circulation Fans

364P Thermal conditioning during the brooder phase may mitigate the negative impact on body weight gain associated with cyclic heat stress in broiler chickens. Aaron Forga^{1*}, Alessandro Rocchi¹, Marcela Cardona¹, Roberto Cuesta¹, Makenly Coles¹, Thaina Barros¹, Juan Latorre Cardenas¹, Guillermo Tellez-Isaias¹, Danielle

Graham¹, Billy Hargis¹, Tom Porter², ¹*University of Arkansas, Fayetteville, Arkansas, United States*, ²*University of Maryland, College Park, Maryland, United States*.

Heat stress (HS) conditions have been shown to hinder body weight gain (BWG) in commercial broiler chickens. In the current study, broiler chicks were exposed to higher than normal brooding temperatures (37°C) to evaluate if thermal conditioning (TC) by early exposure to elevated temperatures could alleviate the reduction in BWG associated with cyclic HS during the grow out phase. Day-of-hatch chicks (n=720) were individually tagged, weighed, and randomly assigned to 6 replicate pens (n=30): thermoneutral (TN); 6h TC; 12h TC; or 24h TC. From day 4-5, brooding chamber temperatures were increased from 32°C to 37°C to induce panting for TC treatment groups for a duration of 6h, 12h, or 24h. Following TC, all groups were maintained at the age appropriate temperature until the beginning of the cyclic HS treatment at day 28. Each group was then divided into 3 replicate pens and subjected to either TN or HS. Individual body weights were recorded at day 0, day 27 (~24h pre-HS), and day 36 (~12 post-HS) to evaluate BWG during the cyclic HS period. From day 28-35, chamber temperatures for HS groups was increased to 37°C for 8h. There were no significant ($P<0.05$) differences in BWG from day 0-day 27 across all groups. HS caused a significant ($P<0.05$) reduction in BWG in all groups as compared to all groups maintained at TN conditions. TC for 6h or 12h had no effect on BWG during cyclic HS. However, BWG in the pens that experienced 24h TC ($716.59\text{g} \pm 9.76\text{g}$) was markedly higher ($P<0.05$) during the HS period as compared to non-TC but HS control ($649.38\text{g} \pm 11.12\text{g}$). Based on these results, exposure to 37°C for 24h from day 4-5 may be an effective strategy to mitigate the negative effects of heat stress on BWG during the grow out period.

Key Words: body weight gain; broiler; heat stress; brooder; thermal conditioning

365P Pulsed Alternating Wavelength System (PAWS) lighting increases growth but not serotonin turnover rates in grow-out Pekin ducks. Sara Tonissen^{1*}, Jenna Schober¹, Victoria Tetel¹, Esther Oluwagbenga¹, Gregory Fraley¹, ¹*Purdue University, West Lafayette, Indiana, United States*.

The overall health, production, and wellbeing of Pekin ducks can be affected by many environmental factors, especially the type of lighting that they are housed under. Artificial light is required to maintain long daylengths to properly sustain growth and fertility in Pekin ducks. However, there is no standard lighting system used in commercial breeder barns, and there is very little data that evaluates the effects of different light systems on Pekin duck production and welfare parameters. The Pulsed Alternating Wavelength System (PAWS) is a novel LED technology that has been introduced in commercial poultry houses. This light system delivers multiple wavelengths of

light to animals and previous studies within our lab have suggested that PAWS may improve the overall welfare of multiple poultry species, including ducks. Past research has shown that reduced serotonin turnover rates in the brain are related to an improvement in the welfare and wellbeing of many poultry species. The goal of the study was to determine the effects PAWS had on brain serotonin turnover rates in breeder ducks. We tested the hypothesis that Pekin ducks housed under the PAWS lights would have lower brain serotonin turnover rates compared to those housed under the control lights. The ducks were placed under the lighting at the day of hatch until they were processed at 30 days of age. Ducks were housed in floor pens (~1200 ducks/pen, N = 4 pens per treatment) with pine shavings and were fed and watered *ad lib* as per industry standards. Body weights and feed intake were monitored weekly. Brain samples were collected on days 0, 7, 14, and 29 from ducks housed under the PAWS or control lights (N = 6 per week, per lighting type). After collection, the brains were frozen on dry ice and stored at -80°C until processed. Brains were microdissected into the dorsal and caudal raphe, the diencephalon, and the telencephalon before being processed using mass spectrometry to measure levels of serotonin and its metabolites. Data were analyzed using 2-way ANOVA for age and lighting treatment with a *post hoc* Fisher's PLSD test. Ducks housed under PAWS lights showed a significant increase in bodyweight by 29 days of age ($p < 0.001$) but had no differences in feed conversion rate compared to controls. Brain analyses showed no differences in the brain serotonin turnover rates between the control and PAWS treatments in any brain area. Our results suggest that PAWS lights may have benefits for production traits, however, a complete understanding of how PAWS lights effect welfare needs further study.

Key Words: light; Pekin duck; welfare; 5-HT

366P Engineering and testing a low-cost air-scrubber to mitigate poultry house emissions. Wendy Attuquayefio^{1*}, James McNaughton¹, Michael Barnas¹, Steve Auman¹, ¹*AHPPharma, Inc., Hebron, Maryland, United States*.

Air pollution, specifically ammonia (NH₃) and particulate matter (PM) 2.5 have been implicated as contributors to respiratory disease in farmers and livestock. Management practices like windrowing can exacerbate the problem by releasing fine particles and ammonia into the atmosphere. Several interventions have been reported in the literature, including vegetated buffers, fan plume deflectors, biofilters, and air scrubbers. Air scrubbers are particularly interesting because they provide an opportunity to produce fertilizer directly on the farm. By reacting exhaust air with acidified water, the mass transfer of NH₃ occurs from gas to the liquid phase—resulting in a product like ammonium sulfate if sulfuric acid is the acidifier, for example. This research aimed to engineer and test a portable, low-cost air scrubber that would reduce emissions from a poultry house. The air scrubber was built using wood and HDPE plastic to capture

the exhaust from a 91 cm square box fan and pass it through a stream of water before being emitted into the atmosphere. A chemical resistant pump recirculated the acidified water containing 1% sodium bisulfate at 7.5 lpm. One Multi Gas Detector (GasLab Plus CM-507, CO2Meter) was placed at the entry and exit of the scrubber to measure the average carbon dioxide (CO₂), ammonia (NH₃), PM 2.5, and PM 10 before and after treatment. The scrubber ran for ten minutes, and the sensors recorded a data point every two seconds (N=300). One single spray and three consecutive sprays were tested. Each spray consisted of a 121 cm square box with three HDPE fan nozzles overlapping a stream of water flowing at 7.5 lpm, which contacts the air prior to entry into the next 121 cm square box. Average NH₃ before the air scrubber was 41 ppm, and after treatment with a single spray, NH₃ was reduced to 15 ppm. After three sprays average NH₃ was 2 ppm. PM 2.5 was reduced from 16 µg/m³ before the scrubber to 8 and 3 µg/m³ after one and three interventions, respectively. The average CO₂ was reduced from 1633 ppm to 791 ppm after a single spray and 401 ppm after three sprays. PM 10 was reduced from 24 µg/m³ before the scrubber to 18 and 4 µg/m³ after one and three sprays, respectively. Based on this research, acid scrubbers are an effective and inexpensive technology to reduce poultry house emissions, especially during windrowing or the general layout period when managing static pressure and ventilation for bird health is not of concern.

Key Words: ammonia; nitrogen; emissions; PM 2.5; air scrubber

367P Growth performance of Delaware and Barred Plymouth Rock pullets through 23 weeks of age. Angela Scott^{1*}, Tuesday Williams¹, Tyler Schaefer¹, Tatijana Fisher¹, ¹*Lincoln University of Missouri, Jefferson City, Missouri, United States.*

Small-flock chicken producers often consider dual-purpose heritage breeds instead of specialized commercial meat or egg strains. Heritage breeds can mate naturally and breed true which can improve on-farm self-sufficiency. Additionally, when compared with modern commercial strains, heritage breeds are said to have improved longevity, better disease resistance, and better tolerance to heat and cold. Unfortunately, these claims are not well validated and little data about the production parameters of heritage breeds is available for interested producers to use to make decisions and develop business plans. The objective of this study was to evaluate the growth rate, feed consumption, and feed conversion ratio of pullets from two heritage chicken breeds: Barred Plymouth Rock and Delaware. These two breeds were selected because they are popular among small-flock poultry producers and are said to be good egg producers. These breeds also present a unique opportunity for small-flock producers to sell sexed chicks because the females can be used to produce sex-link crosses where their offspring can be sexed by color at hatch. Forty-

eight 1-day-old pullet chicks per breed were divided into three replicate groups which were randomly assigned to indoor floor pens with 0.17 m²/bird. At 74 days of age, pullets were moved outside to mobile pasture pens with 0.42 m²/bird. Pens were moved weekly to fresh pasture. Throughout the study, all pullets were provided ad libitum access to a complete chick feed (19% CP), grit, and water. Growth parameters were measured through 23 weeks of age when all pens had begun laying eggs. Statistical analyses were performed as a one-way ANOVA with a threshold of P<0.05. At 160 days of age, the pullets averaged 1893 grams with no significant (P>0.05) difference between breeds. Delaware and Barred Plymouth Rock pullets exhibited similar growth rates (11.7 grams/bird/day), feed intake (66.7 grams/bird/day), and feed conversion ratios (5.7 grams feed/gram gain) over the study period with no significant (P>0.05) difference between breeds. Mortality was low (4%) with no significant (P>0.05) difference between breeds. These results indicate that Delaware and Barred Plymouth Rock pullets have similar production parameters. Therefore, raising replacement pullets of either breed should have similar production costs and producers can consider other factors when deciding between these two breeds for their operation.

Key Words: heritage breeds; alternative production; small flock; replacement pullets; dual purpose

368P Egg production of Delaware and Barred Plymouth Rock pullets through 52 weeks of age. Angela Scott^{1*}, Tyler Schaefer¹, Tuesday Williams¹, Tatijana Fisher¹, ¹*Lincoln University of Missouri, Jefferson City, Missouri, United States.*

Small-flock chicken producers often manage chickens in housing exposed to natural environmental conditions. These producers may use dual-purpose heritage breeds instead of specialized commercial strains because heritage breeds are said to be better suited to these conditions, but published data on production parameters of heritage breeds is lacking. The objective of this study was to evaluate egg production of pullets from two heritage chicken breeds: Barred Plymouth Rock (BPR) and Delaware. These breeds were selected because they are advertised as good egg producers, are popular in small flocks, and are readily available. Forty-eight 1-day-old pullet chicks per breed were purchased March 1st and divided into three replicate groups. The groups were randomly assigned to indoor floor pens with 0.17 m²/bird. While no chicks died in the growth phase, seven Delawares and one BPR were identified as cockerels and removed. At 10 weeks of age, fourteen pullets from each group were moved to mobile pasture pens with 0.42 m²/bird. Mobile pens were moved to fresh grass weekly during the growing season and bedded with pine shavings thereafter. Pullets were provided with ad libitum access to feed (19% CP chick crumble through 20 weeks of age and a 16% layer pellet thereafter), grit, and water throughout the study. Supplemental lighting was not provided. Statistical

analyses were performed as a one-way ANOVA with a threshold of $P < 0.05$. Body weights were similar between breeds throughout. Overall feed intake averaged 101 g/b/d and was similar ($P > 0.05$) between breeds. Mortality was 3.6% with no significant ($P > 0.05$) difference between breeds. There were no significant differences between breeds for average age at first egg (142 days, $P > 0.05$), average weight of first egg (37.2 grams, $P > 0.05$), or overall average egg weight (52.9 grams, $P > 0.05$). Egg production peaked at 48% Hen-Day Egg Production (HDEP) at 27 weeks of age with no significant ($P > 0.05$) difference between breeds. This peak occurred in September with production decreasing each week as daylength shortened. The lowest HDEP (6%, $P > 0.05$) occurred in January at 45 weeks of age with no difference between breeds. As daylength increased in spring, HDEP rose with 42% HDEP achieved in the last week of the study with no significant ($P > 0.05$) difference between breeds. Low HDEP throughout the study was attributed to a combination of genetics, seasonal effects, and egg predation. These results indicate Delaware and BPR pullets have similar egg production parameters through 52 weeks of age when managed under natural conditions. This data should aid small-flock producers when considering these breeds for egg production.

Key Words: egg production; pullets; heritage breeds; small flock; dual purpose

369P Engineering and testing a low-cost dosing system for drinking water lines in poultry houses. Wendy Attuquayefio^{1*}, Michael Barnas¹, James McNaughton¹, ¹AHPPharma, Inc., Hebron, Maryland, United States.

Chickens consume approximately twice the amount of water as feed. Consequently, administering water-soluble ingredients through the drinking water lines can effectively deliver supplemental nutrients to the flock if water quality is maintained. Instead of continuously supplementing the drinking water with additives, dosing for several hours per day can achieve similar efficacy at a lower cost. This research aimed to engineer and test an inexpensive water dosing and sanitation system and then measure *Salmonella* reduction in broilers reared with and without dosing chlorine dioxide (ClO₂). It was hypothesized that broilers challenged with *Salmonella* Heidelberg and dosed with chlorine dioxide (ClO₂) in the water would have fewer *Salmonella* in their gastrointestinal tract (GIT) than broilers reared on standard water only because previous studies have shown that ClO₂ can eliminate pathogens like *Salmonella* by directly damaging the cell wall of microorganisms. Day-old Cobb-500 chicks were randomly assigned to six pens containing 30 birds each. The control group (N=90) received standard water only from 0-42d, while the dosing group (N=90) received five ppm ClO₂ from 6 am-2 pm (8h) daily. Birds were individually weighed each week, and feed consumption was tracked for each pen.

All birds were challenged at 7d and 40-42d with an oral gavage containing 1×10^5 CFU *Salmonella* Heidelberg. At 42d of age, three birds per pen were euthanized, and the small intestine contents were pooled together to create one sample per bird. Then, the cecal contents were similarly pooled for each bird. Next, the empty GIT tissue was homogenized using a sterile blender, and 1.0-gram aliquots of contents and homogenized tissue were cultured using standard plating methods to quantify *Salmonella*. Data were analyzed for a one-way ANOVA using the GLM procedure (SAS 3.8) to determine the effect of treatment on pathogen reduction and live performance. Post-hoc means separation was conducted using Tukey's range test with an alpha of 0.05. There were no differences in live performance between the two treatments ($P > 0.05$). However, the ClO₂ group had zero percent *Salmonella* incidence throughout the GIT contents and tissue, significantly less than the control ($P < 0.05$) with an average of 4.0, 4.1, and 3.6 log₁₀ CFU/g *Salmonella*, respectively, in the small intestine contents, cecal contents, and homogenized tissue. Practical use of this dosing system could effectively control poultry pathogens and biofilm accumulation in the water lines while reducing the total chemical usage, improving sustainability. Future studies will explore shorter dosing periods and different water additives.

Key Words: biofilm; disinfectant; *Salmonella*; water quality; chlorine dioxide

370P Effect of elevated RH on broiler performance when fed nicarbazin. Kristin Miles^{1*}, Michael Czarick¹, Garret Ashabranner¹, Brian Fairchild¹, ¹University of Georgia, Athens, Georgia, United States.

The anticoccidial, nicarbazin, is known to cause a reduction in performance and impairs the ability of poultry to cope with high ambient air temperatures. Heat stress can be intensified by increased relative humidity (RH). The purpose of this study was to determine the effect of nicarbazin fed for 28 days on performance of broilers at two levels of RH: 35-50% and 65-75%. Three trials were conducted with 32 pens for Trials 1 and 2 and 48 pens for Trial 3 with 27 birds per pen. Chicks were assigned to one of four treatment groups: 30-50% RH, fed a control diet, 30-50% RH, fed a nicarbazin diet, 65-75% RH, fed a control diet, and 65-75% RH, fed a nicarbazin diet. The birds were reared for 35 days during which the nicarbazin diet was fed for the first 28 days then switched to control diet for the remainder of the study. The treatment feed contained nicarbazin at a rate of 125 ppm and 99 ppm for the starter and grower diets respectively while control diet did not contain nicarbazin. A coccidiosis challenge was not administered for this study and birds were necropsied at 21 days and evaluated for coccidiosis. No evidence of coccidiosis was found. Data were analyzed using a split plot ANOVA while separating means with Student's *t* ($P < 0.050$). Nicarbazin treated birds had lower body weights and feed consumption than those fed the control diet for the

five-week study ($P < 0.003$). There was a decrease in weight gain for the nicarbazin treatment group for the first four weeks but was not decreased after nicarbazin was removed during Week 5 ($P < 0.002$). FCR was increased in the nicarbazin treated broilers compared to those fed the control diet during the four-week treatment period ($P < 0.001$). During the fifth week when all birds were fed the control diet, the birds previously fed nicarbazin had a lower FCR than the control fed birds ($P < 0.023$). No significant differences in weight and gain were observed between the RH treatments or the interaction between RH and diet. 65-75% RH resulted in lower FCR during the first week in two of the three trials ($P < 0.016$). The interaction between RH and diet showed a significant difference in FCR for Weeks 3 and 4 in Trial 1 and Week 3 of Trial 3 ($P < 0.046$). There were numerous significant differences in rectal temperature (0.1°C - 0.2°C) for diet and interactions during two of the three trials ($P < 0.046$), however these 0.2°C or less differences were not considered to be biologically meaningful. Treating with nicarbazin caused a decrease in five-week broiler performance in the absence of a cocci challenge. Elevated RH did not depress overall performance or exacerbate the negative effects on five-week performance observed with nicarbazin treatment.

Key Words: feed conversion; anticoccidial; relative humidity

371P Impacts of on-farm water sanitation practices on microbial levels in poultry water system and efficacy of sodium hypochlorite-based product on *Salmonella Enteritidis*. Pramir Maharjan^{1*}, Agnes Kilonzo-Nthenge¹, Tobenna Aniume¹, Ashesh Basnet¹, Morgan Roberts¹, Samuel Nahashon¹, Susan Watkins², ¹Tennessee State University, Nashville, Tennessee, United States, ²University of Arkansas, Fayetteville, Arkansas, United States.

Microbial water quality of poultry water supplies can be correlated to bird health, livability, and overall production. Two separate field studies were conducted to understand microbial hygiene of poultry waterlines based on the water sanitation practices. In the first study, two farms were selected-Farm A that did not treat poultry drinking water supplies; farm B treated water during flock grow-out period. Prevalence test for specific pathogens- *Salmonella*, *E. coli*, *Campylobacter*, *Listeria*, and *Staphylococcus* were performed for both farms by collecting swab samples post bird harvest ($n=4$ lines, each farm) from waterlines and plating in specific growth media. In another study, a broiler farm that had four barn units that treated water ($\text{Cl} = \sim 1$ ppm) during flock grow-out period and flush waterlines between flocks using concentrated chlorine solution (>1000 ppm) was selected. Swab samples (one from each barn, $n=4$; each sampling occasion) were collected at three occasions-before flushing, after flushing and at the end of grow-out period (d 42) to understand the biofilm growth rate. Additionally, a separate benchtop study was conducted to understand the efficacy of a commonly used poultry

drinking water sanitizer product (sodium hypochlorite, 8.25 %) against *Salmonella Enteritidis* (SE) mimicking application rate of the product as used in field practice. *Salmonella Enteritidis* broth culture (2.2×10^8 cfu/ml) was seeded to test water (12.8ml) at three inoculum pathogen sizes (0.5ml, 1 ml and 1.5ml) and then treated with the product (100ul). Samples were plated in triplicate at three occasions-before treatment, and post treatment (5 min and 3 hr). Results from the prevalence study showed that the farm A and farm B were positive for *Listeria* and *Staphylococcus*, whereas found negative for other species tested. The findings from the broiler farm swab sample evaluation for biofilm showed the aerobic plate count of $>4 \log_{10}$ cfu/ml before flushing, and then dropped to $< 1 \log_{10}$ unit ($P < 0.05$) after flushing. The bacterial count went up to $> 4 \log_{10}$ units on d 42 compared to initial count at post flushing ($P < 0.05$). The efficacy test showed that the product was effective post 5 min (0 cfu/ml) for the lowest inoculum of SE seeded whereas the other two inoculum sizes had $> 3 \log_{10}$ cfu/ml of SE. There were no SE counts observed at 3 hr post treatment for all inoculum sizes. The results indicated that even treated farm were vulnerable to biofilm growth in poultry water system over time, and the product efficacy could be affected by pathogen (SE) load in water supplies.

Key Words: biofilm; sanitizer; efficacy; water supplies; microbial levels

372P Evaluation of formaldehyde application in commercial hatcheries on chick gut microbiota. Brett Meisinger^{1*}, Craig Coufal¹, John Carey¹, ¹Texas A&M University, College Station, Texas, United States.

Commercial hatcheries are a key part of the poultry industry, and the quality of chicks produced can influence flock health throughout production. Hatchery sanitation procedures, such as formaldehyde application in hatcheries, has been shown to decrease airborne microbial counts. However, the impact of formaldehyde application in hatcheries on the microbiota of the chick intestinal tract is also of great interest. The objective of this experiment was to evaluate the impact of formaldehyde application in hatcheries compared to hatcheries without formaldehyde application on embryo and chick intestinal tract microbiota on day 18 of hatch. Two hatcheries in a commercial hatchery were selected to receive eggs from the same breeder flock. Five eggs containing viable embryos were randomly selected on day 18 from incubators (prior to transfer) and day 19 from hatcheries. Following normal hatchery procedures, eggs were transferred and processed through in-ovo injection on day 19. Hatched, live chicks were randomly selected on days 20 and 21 from the formaldehyde-treated and untreated hatcheries. On each day, embryos or chicks were euthanized and the intestinal tract was aseptically removed and placed in a sterile Whirl-pak bag. Sterile PBS was added at 4 times the weight of the intestinal tract, and the sample was thoroughly homogenized. Serial dilutions of the

homogenized intestinal tract sample were plated on Tryptic Soy Agar, Mannitol Salt Agar, Bile Esculin Agar, EMB and Egg Yolk Agar medias to enumerate total aerobic and anaerobic plate counts, *Staphylococcus aureus*, *Enterococcus* and Group D *Streptococcus*, *E. coli* and other gram negatives, and *Clostridium* spp., respectively. It is hypothesized that there will be no difference in the survey of chick intestinal microflora attributable to formaldehyde application. Plate counts for each day were analyzed separately using a Generalized Linear Model (GLM). There were no significant differences for intestinal tract counts on any media on any day for samples obtained from the formaldehyde-treated and nontreated hatchers. Additional trials are needed to support the current findings.

Key Words: intestinal tract; hatching eggs; Hatchery; formaldehyde; microbial contamination

373P The effect of relative humidity on litter moisture and foot pad dermatitis of broilers fed with and without nicarbazin. Garret Ashabranner^{1*}, Kristin Miles¹, Michael Czarick¹, Brian Fairchild¹, ¹*University of Georgia, Athens, Georgia, United States.*

Litter moisture is known to have a direct correlation with foot pad dermatitis. In addition, relative humidity (RH) can influence the moisture content of litter. All feedstuffs, supplements, and medications can affect litter moisture by increasing the retention or excretion of nutrients and water during broiler feed digestion. The objective of this study was to determine how RH affects litter moisture and foot pad dermatitis in birds fed with and without nicarbazin. A 35 day study with eight pens of 27 Ross 708 broiler chicks were placed in 10 identical rooms. Half the birds in each room were fed a control diet where the other half were fed a nicarbazin treated feed with a concentration of 125 ppm and 99 ppm for the starter and grower diets respectively. On Day 28 all birds were fed the same control diet absent of nicarbazin. Three rooms were subject to 35-50% RH and three rooms subjected to 65-75% RH. Litter moisture and foot pad dermatitis scores were taken on Days 21, 28, and 35. Approximately 100g of litter was collected, weighed, and dried in an oven at 75°C. The change in litter weight after drying was used to calculate percent moisture content. Foot pad dermatitis scores were examined using a three point scale (0-2) (Bilgili et al., 2009). All data were analyzed using ANOVA with diet and RH as the main effects, differences were considered significant at ($p < 0.05$). Litter moisture was significantly higher in the 65-75% RH treatment on Days 21 and 35. Diet only had an effect on litter moisture on Day 35 where the nicarbazin treatment pens had significantly lower litter moisture content. This period was when the nicarbazin treated birds were fed a control finisher diet on Days 28-35. The change in diet may have had a greater effect than the nicarbazin itself on the litter moisture as diet changes have been shown to decrease water consumption. Foot pad dermatitis scores trended

higher in the 65-75% RH but were not significant. On Days 28 and 35 there were significantly more scores of 0 in the low RH rooms while the 65-75% RH had significantly higher scores of 1. Relative humidity treatment did not significantly affect the occurrence of lesion score of 2 throughout the study. Diet had no significant effects on foot pad dermatitis throughout the study. The results of this study support the findings of other literature that higher RH levels increase litter moisture inadvertently increasing the occurrence of foot pad dermatitis. These results also suggest that water excretion might have decreased due to the feed change.

Key Words: foot lesions; paw quality; husbandry conditions; moisture content; environmental conditions

374P Response of Ross 308 breeder hens to different levels of protein and/or essential amino acids. Léa Verrière^{1*}, Carole Margetyal¹, Olivier Gestin², Marie-Laure Godde², Claire Launay¹, ¹*ADM Animal Nutrition Neovia, Château-Thierry, France,* ²*ADM Animal Nutrition SERMIX, Saint-Nolff, France.*

Nutritional trials on broiler breeder hens are not frequent; their amino acids requirements are consequently still not fully defined and deserve to be unravelled. A study was thus conducted to evaluate the effect of crude protein (CP) level, depending on the essential amino acids (EAA) contents. A total of 140 Ross 308 broiler breeder hens were placed in individual cages and distributed in a completely randomized design among 4 treatments, with 35 replicates each. Treatments were provided from 46 to 53 weeks of age after a 5 weeks adaptation period. The experimental treatments were composed by 2 levels of total protein, namely 12% and 13% CP, associated with either high (EAA+: 0.52% digestible lysine, 0.35% digestible methionine, 0.47% digestible threonine, 0.47% digestible valine) or low (EAA-: 0.46% digestible lysine, 0.26% digestible methionine, 0.36% digestible threonine, 0.46% digestible valine) essential amino acids levels in the feeds. Feed distribution was restricted to 140 g/d/hen and then reduced to 139 g/d/hen, according to the mean weight of the flock. The feed consumption of each experimental unit was measured on a weekly basis. Corresponding laying performance (laying rate, egg weight, egg mass produced, feed conversion ratio per g of egg mass and per eggs, abnormal eggs) were assessed. Breeder hens were weighed at the beginning and at the end of the trial to assess the potential impact of treatments on body weights. Data were firstly subject to a one-way ANOVA using a repeated measures model (gls) and means with significant differences were separated by Tukey's HSD test at $P < 0.05$. In addition, the respective effects of CP level, EAA levels and their interactions were further explored using a 2-way ANOVA. The slopes of laying rate decrease during the trial were compared among the 4 treatments. It showed that laying rate persistency benefits from cumulated effects of higher CP and EAA contents. At 13% CP, it clearly seems that egg weight isn't

affected by the level of EAA (63.6g with EAA+, 63.4g with EAA-). At 12% CP, EAA+ benefits more to the laying rate than to the egg weight, which conduces to an egg mass produced improved with EAA+ (+1.7 point, $P = 0.01$) whereas the effect at 13% CP is only marginal. Feed efficiency follows the same pattern, with a significant improvement due to EAA+, at 12% CP (-0.11 , $P < 0.05$). Finally, low protein and low EAA profile have cumulated negative effects on body weight maintenance. Overall, these results show how important it is to ensure a good balance in the global amino acids supply. At the same time they give insights on the way each criteria can benefit, either from CP or EAA modulation.

Key Words: amino acids; protein; breeders; Ross 308

375P Impacts of reduced crude protein diets in broilers supplemented with feed-grade amino acids on growth performance, digestibility of nutrients, nitrogen utilization and footpad health. Roshan Adhikari^{1*}, Mitch Davis², Reed Dillard², Jason Lee¹, Adam Davis², ¹*CJ Bio America, Downers Grove, Illinois, United States*, ²*The University of Georgia, Athens, Georgia, United States*.

With the increasing cost of feed ingredients, the availability of feed grade forms of Val, Ile, and Arg allows nutritionists to utilize those to meet limiting AA requirements while reducing CP and diet cost. The objective of the study was to evaluate the effect of a reduction in dietary CP on performance, digestibility, and nitrogen content in broilers fed diets with optimized AA profile based on ideal protein concept meeting the 4th, 5th, 6th limiting AA requirements through the addition of feed grade amino acids. A total of 2,160 day-old male Ross 708 broilers were assigned to five dietary treatments distributed randomly to 80 floor pens. Diets utilizing corn, SBM, DDGS and supplemented Met, Lys, Thr in crystalline form were formulated as T1, T2, T3, and T4, and were made by adding Val, Arg and Ile respectively in the crystalline form to reduce dietary CP but meet the minimum requirement of 4th, 5th, and 6th amino acid. T5 was a similar diet to T4 except added crystalline glycine to match dGly+Ser:dLys as in T1. Birds were fed a four-phase feeding program; starter (0-14), grower (14-28), finisher (28-42), and withdrawal (42-49). Bodyweight and FCR were measured at the end of each dietary period. On d 49, footpads were visually scored for lesions on all birds and a composite litter sample was taken from each pen to determine the nitrogen content. At the end of the experiment, 7 broilers per pen were selected and processed. Data were subjected to ANOVA according to the General Linear Model (GLM). Tukey's multiple-comparison procedure was used to detect significant differences among dietary treatments. For T1-T4, there were no differences in body weight gain or FCR from d 0-49, or in total white meat, wing or leg quarter yields. Litter nitrogen was significantly lower in birds fed T4 than T2. There was a significant improvement in food pad score in birds fed T4 compared to T1, T2, or T3. Apparent ileal digestibility of starch, nitrogen

and gross energy was significantly higher in birds fed T4 compared to T1, T2, or T3. Increasing Gly in T5 lowered body weight gain and increased feed to gain but improved footpad and litter score compared to T4. These data demonstrate the benefits of reducing dietary CP on nutrient digestibility, foot pad health, and nutrient excretion without compromising final flock performance or meat yield.

Key Words: Broilers; Arginine; Valine; Isoleucine; Low CP

376P Assessment of broiler responsiveness to varying levels of digestible histidine for a 14-35d grower period.

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The objective of this study was to assess current male broiler responsiveness to varying levels of histidine in practical corn- soy diets between 14-35 days of age. Male Cobb 500 broiler chicks were placed in floor pens (56 pens with 12 birds per pen) measuring 3' x 4' with new pine shavings and reared in a solid-walled, tunnel ventilated growout facility where access to feed and water were provided *ad libitum*. Prior to the experimental period, all birds were fed a common starter diet from 1 -7 days. On day 8, all birds were fed a diet formulated to be marginally deficient in histidine (18% CP, 3088 kcal/kg ME, 0.30% dig His) to allow for the depletion of histidine containing compounds and prevent the overestimation of requirement values. The pretest diet was fed from 8 -13 days. Upon conclusion of this period, all pens were weighed yielding an average bird weight of 446 grams. On day 14, pens were randomly assigned to one of 7 treatments with 8 replicate pens per treatment. Pen was considered the experimental unit, and treatments were assigned to pens in a randomized complete block design with pen location as the blocking factor. A basal diet (16.75% CP, 3085 kcal/kg ME, 1.12% lysine) was formulated to meet or exceed all Cobb 500 broiler recommendations apart from histidine, which was calculated at 0.33% digestible. Successive additions of 0.03% of L-histidine were included at the expense of a filler (equal mixture of washed sand and cellulose) and capped at 0.49% digestible histidine. A positive control diet (19% CP, 3085 kcal/kg ME, 1.12% lysine) was formulated to match the His titration diet with 0.40% His where the two performed similarly. Data were analyzed with SAS 9.4 using the PROC GLM and REG features. Linear and quadratic effects were not observed on any performance parameters based on $P \leq 0.05$. Collective pen estimates produced means for ABWG, AFI, and FCR of 1849 grams, 3122 grams, and 1.7072 respectively. Based on these results, it appears that digestible histidine levels from 0.34% - 0.49% do not influence live performance of broilers from 14 -35 days.

Key Words: amino acids; broiler; nutrition; requirement; histidine

377P A systematic review on the role of functional amino acids on growth performance and intestinal integrity in broiler chickens exposed to coccidiosis.

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Avian coccidiosis is a parasitic disease caused by the protozoa *Eimeria* and is responsible for significant economic losses to the poultry industry. As certain amino acids (AA) can serve as source of energy or building blocks of functional proteins such as mucins, immunoglobulins for the intestinal mucosa. As such nutritional intervention of these AA may be key in preserving or restoring gut health of broiler chickens during an intestinal disturbance such as coccidiosis. A systematic review was performed to summarize the effects of functional AA supplementation on growth performance and intestinal integrity in broiler chickens subjected to experimental coccidiosis. An initial search on Google Scholar and in the University of Guelph Omni database was done to retrieve articles related to the subject based on the article titles. The eligibility criteria for the articles were: 1) published in a peer-review journal between 2000 and 2021, 2) conducted in broiler chickens challenged with *Eimeria* (single spp. or multiple) 3) supplementation of at least one AA, and 4) at least one parameter of growth performance or intestinal integrity measured. A database of 33 articles was built and data extracted following an adapted methodology where percentage difference between the supplemented and control group was recorded only when the comparison was statistically significant within each experiment. Evidence was considered strong if at least 66% of studies reported consistent results for a specific parameter, moderate if at least 50%, or inconclusive if less than 50%, given that the AA was tested in at least 3 different studies. Most of the selected studies focused on four AA: Arginine (**Arg**), Glutamine (**Gln**), Threonine (**Thr**), and Methionine (**Met**), ranging from 0.2% to 30% above recommendation. The data showed that on-top Arg, Gln, Thr, and Met supplementation had inconclusive evidence for improving body weight gain, feed intake, and feed conversion ratio in starter, grower, and finisher phases. While Arg and Gln supplementation was inconclusive for improving villi height, Thr supplementation showed moderate evidence for increasing (average of 16.92%) jejunal villi height and improving (average of 13.18%) jejunal villi height to crypt depth ratio. However, for most of the studied parameters, no clear conclusion could be drawn based on this methodology due to either a lack of data and/or the variability in the response criterion. Thus, warranting more studies to

quantify the effects of these functional AA as potential prospect for coccidiosis management in broiler chickens.

Key Words: broiler chickens; coccidiosis; gut health; immunity; functional amino acids

378P Growth performance and apparent retention of components in broiler chickens fed corn-soybean-based diets with sunflower meal and wheat middlings pretreated with fiber degrading enzymes. Felix Njeri^{1*}, Elijah Kiarie², Charles Gachui¹,
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Pre-treating fibrous feedstuffs with fiber degrading enzymes (FDE) may improve utilization in poultry. We investigated the impact of the inclusion of pretreated sunflower (TSM) and wheat middling (TWM) in a corn-soybean meal (CSBM) on growth performance and apparent retention (AR) of components. A total of 288 Ross-708 d old male broiler chicks were placed in cages (6 birds/cage) based on body weight (BW) and allocated to 6 diets in a completely randomized design giving 8 replicates per diet. The diets were: 1) PC, a CSBM, positive control (PC), 2) NC, PC plus untreated sunflower meal (USM) and wheat middling (UWM), and 3) 4 test diets in which USM and UWM were replaced with TSM and TWM at 25% (N25), 50% (N50), 75% (N75) and 100% (N100). For pre-treatment, each feedstuff was mixed with 1% of FDE in a ratio of 1:2 wt/wt for feedstuff: water, incubated for 24 hours at 40°C and oven-dried at 60-65°C for 72 hrs before feed preparation. Diets were formulated for two phases (starter, d 0-21) and finisher (d 22-42). The negative control and test diets were formulated to be low nutrient-dense by having a lower AMEn and crude protein compared with PC. All diets had titanium dioxide (0.3%) for AR determination. Birds had free access to feed and water. The BW, feed intake (FI), and mortality were recorded. Excreta was collected and frozen (-20°C) per cage from d18 through to d20 for the starter phase and from d39 to d41 for the finisher phase. The data were subjected to a one-way ANOVA using PROC GLIMMIX of SAS 9.4 and Lsmeans separated using the Tukey test ($P < 0.05$). Pre-treatment increased soluble protein (SP) by 5.08%, decreased both neutral detergent protein (NDF-P) and neutral detergent fiber (NDF) by 1.33% and 10.5%, respectively, in sunflower. Compared to UWM, TWM had 3.44% less SP while both NDF-P and NDF were greater by 1.63% and 3.8%, respectively. The BW and BW gain (BWG) of N75 and N100 were less than PC ($P \leq 0.001$) throughout the trial. Feed intake was not different during the starter, finisher, and overall period ($P \geq 0.217$) relative to PC and NC. Also, feed conversion ratios were not different throughout the study ($P \geq 0.151$), except for N75 during the starter phase ($P = 0.005$) when compared with PC and NC. The test diets did not affect apparent crude protein retention ($P = 0.07$); there was decreased apparent NDF on d21 and d42 ($P \leq 0.001$) and apparent gross energy retention on d21 and d 42 ($P \leq 0.001$) compared to PC. In

conclusion, although pre-treatment altered fiber and fiber bound protein in test feedstuffs, there was no improvement in growth performance and components retention in broiler chickens.

Key Words: Performance; Apparent retention; Fiber degrading enzymes; Broiler birds; Soluble protein

379P Performance of 42-day broilers fed HiPhorius™ and HiPhos™ phytases at standard and superdose levels. Nelson Ward^{1*}, Carrie Walk², Randolph Mitchell³, ¹DSM Nutritional Products, Ringoes, New Jersey, United States, ²DSM Nutritional Products, UK, Heanor, United Kingdom, ³Perdue Foods LLC, Salisbury, Maryland, United States.

HiPhorius is a new variant of HiPhos phytase with a significant increase in stability at low intestinal pH and in the presence of pepsin. This results in more phytase activity when phytic acid is in the free form and available for dephosphorylation. This trial compared HiPhorius with its predecessor HiPhos in a low non-phytate phosphorus (nPP) and low calcium (Ca) corn/SBM pelleted feed for broilers over a 42-day period. Standard doses of HiPhorius (600 FYT/kg) and HiPhos (1000 FYT/kg) to replace 0.15% nPP were compared to superdose levels (HiPhorius 1500 FYT/kg; HiPhos 2500 FYT/kg). Day of hatch, Ross x 708 male broiler chicks (n = 4,608) were randomly placed at the rate of 48 birds/pen in 96 pens with built up litter. With 16 replicates/diet, six treatment diets were fed in three phases: Starter (S, d1-14), Grower (G, d15-28), and Finisher (F, d29-42). The positive control (PC) was formulated to be nutritionally adequate as noted: S, 0.50% nPP & 0.95% Ca; G, 0.43% nPP & 0.86% Ca; F, 0.35% nPP & 0.70% Ca. The negative control (NC) in all phases was formulated to contain 0.15% less nPP and Ca. HiPhorius or HiPhos (FYT/kg) was added to NC to create the remaining 4 treatments: NC + HiPhorius 600, NC + HiPhos 1000, NC + HiPhorius 1500, and NC + HiPhos 2500. Tibiae from 3 birds/pen were obtained on d25. Data were analyzed as a randomized block design with means separated by Tukey-Kramer all-pairs test. Mortality did not differ across treatments. Feed intake over the 42-day period was greater (P<0.05) for all phytase treatments when compared to the NC, but not different from the PC. On d42, body weight (BW) of birds fed HiPhorius 600 and HiPhos 1000 was improved (P<0.05) over birds fed the NC by 3.6% and 2.8%, respectively, and similar to birds fed the PC. HiPhorius 1500 and HiPhos 2500 improved (P<0.05) BW by 5.5 and 5.3%, respectively, compared with birds fed the NC and 3.7% and 3.5% heavier (P<0.05) than birds fed the PC. Feed/gain (FCR) for HiPhorius 600 and HiPhos 1000 did not differ from PC or NC, whereas both phytase superdose levels improved (P<0.05) FCR versus PC birds. Relative to the standard phytase dose, the combined superdose levels elevated (P<0.05) BW by 2.1% and improved (P<0.05) FCR by 1.8%. The 25-day tibia ash % (TA) for NC was lower (P<0.05) than PC. TA was higher

(P<0.05) in all phytase treatments compared to NC, while the TA for both HiPhorius groups was higher (P<0.05) than respective HiPhos groups. HiPhorius at lower FYT/kg of feed was as effective, or more so, than HiPhos in broiler diets from hatch to d42. Furthermore, superdose levels of both phytases effectively compensated for the low nPP and low Ca levels, while significantly improving BW and FCR.

Key Words: broilers; tibia; phytase; HiPhorius; HiPhos

380P Effects of low nutrient corn-soy diet and enzyme supplement on the performance and eggshell quality of laying hens. Tuoying Ao^{1*}, Anthony Pescatore¹, Marquisha Paul¹, Daniel Graugnard¹, Rebecca Delles¹, Mike Ford¹, Ronan Power¹, ¹Alltech-University of Kentucky Nutritional Research Alliance, Lexington, Kentucky, United States.

The purpose of the current study was to investigate the effect of dietary supplementation of enzyme in corn-soybean meal diet with low nutrient density on the production performance and eggshell quality of laying hens during 44-wk of production. Allzyme® Spectrum, an naturally fermented product with multiple enzyme activities including carbohydrase and phytase was used. Dietary treatments included: 1) corn-soy diet having a commercial equivalent nutrient level; 2) corn-soy diet having 0.15% less Ca and available P compared to diet 1 (Low Ca & P diet) + commercial phytase at 250 FTU/kg; 3) Low Ca & P diet + 0.02% Allzyme® Spectrum; 4) Low Ca & P diet having 90 kcal/kg less ME + 0.02% Allzyme® Spectrum. Six replicate units of six Hy-line brown layers at 16 weeks of age were randomly assigned to each of the four dietary treatments and were raised for 44 weeks. The layers were housed in layer cages with two birds per cage (25 x 41 cm) and photo-stimulated based on the management guide from Hy-line. Six eggs from each unit at 8-wk intervals were randomly collected for the determination of egg weight and eggshell quality. Data were subjected to ANOVA for completely randomized design to identify treatment effects and LSD was used to determine mean differences. Hen-day egg production (mean = 89.4%) and feed intake (mean = 108.5 g/bird) for 44-wk period were not affected by dietary treatments. Average egg weight (mean = 59.7 g), egg breaking strength (mean = 4.51 kg) and per cent shell (mean = 10.2%) did not differ among treatment groups during the 44-wk of egg production. Study results indicated that a corn-soybean meal diet with reduced ME, Ca and available P and supplemented with Allzyme® Spectrum can be used to feed layers during peak production without affecting production performance, egg weight and shell quality.

Key Words: enzyme; production performance; eggshell quality; Layer; reduced nutrients

381P A novel canola meal-derived prebiotic enhanced gut health and performance of broiler chickens. Yanxing (Stella) Niu^{1*}, Anna Rogiewicz¹, Lan Shi¹, Rob Patterson², Bogdan Slominski¹, ¹University of Manitoba, Winnipeg,

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The advanced enzyme technology was used to develop a novel prebiotic containing product of enzymatic hydrolysis of canola meal (CM) fibre (ECMS). A carefully selected blend of pectinase, xylanase, protease, and invertase was used to release bioactive components of CM fibre, and their prebiotic effect on growth performance, nutrient digestibility, and gut ecology of broiler chickens was evaluated. Ross 308 broiler chickens were assigned to 3 dietary treatments in a completely randomized design, each treatment consisting of 8 pens of 6 birds, and fed corn-SBM control (C), Low ECMS (C+1g/kg ECMS), and High ECMS (C+20 g/kg ECMS) mash diets from 1 to 14 d of age. All the statistical analysis was conducted by the SAS program. Means were separated by Tukey's honestly significant difference. The results showed that birds fed a High ECMS-containing diet had significantly higher feed intake and body weight gain when compared with C and Low ECMS treatment ($P<0.05$). The relative abundance (analyzed using the $2^{-\Delta\Delta CT}$ method by normalizing the expression of the target bacterial groups to that of total eubacteria and calibrating the target bacterial groups in C treatments) of *Lactobacillus spp.* was higher in digesta of birds fed Low and High ECMS compared to birds fed C diet, i.e., 39.7 and 80.31 vs. 1.05 in ileum and 18.0 and 59.6 vs. 1.1 in ceca, respectively. The relative abundance of *Enterococcus spp.* in ileal and cecal digesta of birds fed ECMS diets was also higher than in C treatment. Increased total short chain fatty acids (SCFA), volatile fatty acids (VFA), and lactic acid contents in ileal digesta of birds fed ECMS supplemented diet when compared with the C treatment was observed ($P<0.05$). There was no treatment effect on the pH of ileal and cecal digesta, sialic acids secretion, and apparent total tract digestibility of NDF and nitrogen. Overall, the ECMS improved the growth performance of broiler chickens and stimulated the growth of beneficial bacteria. Research demonstrated that enzymatically modified CM acquired prebiotic properties and can exert health benefits for broilers by improving the intestinal environment.

Key Words: broiler chicken; gut health; multi-carbohydrase; canola meal-derived prebiotic (ECMS)

382P The effect of Xylamax on growth performance, gut health, and carcass quality of broilers fed reduced-energy sorghum diets. Yun-mei Lin^{1*}, Sandra Rodrigues¹, Ernesto González², Carlos Coello³, José Menocal², ¹BioResource International, Durham, North Carolina, United States, ²Universidad Michoacana de San Nicolás de Hidalgo, Michoacán, Mexico, ³Universidad Nacional Autónoma de México, Mexico City, Mexico.

NSPase enzymes have become integral parts of commercial poultry feed for enhancing nutrient utilization and optimizing the gut health of birds, thereby promoting growth performance, and reducing the overall cost of

production. The objective of this study was to investigate the efficacy of Xylanase (Xylamax, BRI) on performance, gut health, and carcass quality of broilers fed reduced-energy sorghum. 1350-day-old mixed-sex Cobb 500 chicks were randomly distributed to three treatment groups with 9 replicates of 50 chicks each and grown to 42 days of age. The treatment diets were fed *ad libitum*: Positive control (PC), formulated with the standard metabolizable energy (ME); Negative control (NC), ME reduction of 130 kcal was consistent throughout all diet phases; Xylamax (XM), xylanase supplemented to NC at 100g/MT. Parameters measured included body weight, feed intake, intestinal lesion score, histomorphology, and carcass quality. All parameters were analyzed using one-way ANOVA and means were compared by Tukey test ($p<0.05$). Data was reported as least square mean (LSM) \pm standard error of the mean (SEM). Xylamax treatment significantly increased ($p=0.001$) 42d BW compared to the NC. FCR on the final day was also improved following the same trend. For histomorphology, PC had a significantly longer ($p=0.05$) ileum villus height compared to NC, followed by xylanase treatment had an intermediate ileum villus height. In villus density, Xylanase treatment had a significantly higher density which is also reflected in the villi surface area. The villi surface area per μm^2 represents the most important value of the intestinal morphology status; PC and Xylamax demonstrated significantly higher ($p=0.005$) values versus NC. There was no significant difference observed in other parameters measured. In this trial, Xylamax delivered statistically equal 42d bodyweight with the birds fed the standard energy level in the PC, while saving feed cost by replacing 130 kcal/kg energy. Xylamax demonstrated its efficacy on growth performance and showed a significant improvement in gut morphology compared to NC and numerically better than PC while maintaining the quality of carcass in 130 kcal/kg energy-reduced sorghum diets.

Key Words: NSP; Xylanase; Sorghum; Gut Health; Carcass Quality

383P Evaluation of corn kernel hardness, drying temperature and stimbiotic supplementation on broiler carcass yield, cut up parts and breast meat quality. Joaquin Cabanas-Ojeda^{1*}, Jhon Mejia-Abaunza¹, Paula Lozano-Cruz¹, V.L. Aragao- Neto¹, Muhammad Ali¹, Maria Alfaro-Wisaquillo¹, Gustavo Quintana-Ospina¹, Lina Peñuela-Sierra^{1,3}, Gilson Gomes², Edgar Oviedo-Rondón¹, ¹North Carolina State University, Raleigh, North Carolina, United States, ²AB Vista, Plantation, Florida, United States, ³Universidad del Tolima, Ibagué, Tolima, Colombia.

Variability in corn energy, nutrient and NSP composition due to endosperm hardness and post-harvest drying temperatures (DT) has been observed. Recent studies found beneficial effects of dietary stimbiotic (STB), xylanase+fermentable oligosaccharides, supplementation on broiler performance, carcass yield, and meat quality. This study evaluated the effects of kernel hardness corn

varieties (CV), DT, and supplementation of a STB (Signis[®] AB Vista, Marlborough, UK) on broiler carcass yield, cut up parts, and meat quality. The average (AE) and hard endosperm (HE) CV were planted under similar conditions. At harvest, each variety was split into two groups to be dried at 35 and 120°C. Eight treatments resulted from a 2x2x2 factorial arrangement with two kernel hardness, two DT, and the inclusion of STB (100 g/ton). A total of 1,920 Ross 708 male day-old chicks were placed into 96 floor pens and fed *ad libitum* amounts of starter (900 g/chicken), grower (2,000 g/chicken) and finisher (*ad libitum*) pellet diets up to 42 d. Two broilers per pen were selected and processed at d 43. Data were analyzed in a randomized complete block design. Three-way interaction effects ($P<0.05$) were observed only for myopathy scores (SC). Carcass yield was not affected ($P>0.05$) by treatments. An interaction between CV*DT was observed ($P<0.05$) on breast meat and *Pectoralis minor* yield, and drip loss. Breasts yield and drip loss were higher for broilers fed HE when corn was dried at 35°C. *P. minor* yield improved in broilers fed AE corn when dried at 120°C. *P. major* yield increased (>0.55 % points) in chickens fed diets with any corn dried at 35°C ($P<0.05$), HE corn independently of DT ($P=0.054$), or diets supplemented with STB ($P=0.064$). An interaction effect of CV*STB was observed for 24 h pH. pH increased with STB supplementation only in the HE CV. Breast meat yellowness (*b*) increased in the HE corn ($P<0.001$) and when dried at 120°C ($P<0.05$). Cook loss was not affected ($P>0.05$) by treatments. Three-way interaction effects were detected for wooden breast (WB) and white stripping (WS). Broilers fed diets with average corn dried at 120°C had 10 times less fillets with WB SC2, but higher WB SC, 2.4 times more WS SC3 when supplemented with STB. While, when dried at 35°C the WB SC2 doubled, WS SC1 increased 4 times, and WS SC3 decreased 1.8 times in broilers fed diets with STB. In broilers fed diets with STB, the spaghetti muscle occurrence ($P=0.053$) doubled in broilers fed diets with AE corn, but it was 5.3 times higher in broilers with HE corn. In conclusion, CV and DT affected breast meat yield and some meat quality parameters, and stimbiotic improved breast yield which might also in turn influenced the incidence of myopathies.

Key Words: corn; Broilers; xylanase; kernel hardness; xylo-oligosaccharides

384P The interaction between 3 phytate phosphorus levels and 4 dose levels of a novel consensus bacterial 6-phytase variant in broilers through 42 days of age. Eric Sobotik^{1*}, Gabrielle House¹, alfred dimas¹, Abiodun Bello², Yueming Dersjant- Li³, gregory archer¹, ¹Texas A&M University, College Station, Texas, United States, ²Danisco Animal Nutrition - IFF, Wilmington, Delaware, United States, ³Danisco Animal Nutrition & Health - IFF, Oegstgeest, Netherlands.

This study evaluated the interaction between phytate phosphorus (PP) and a novel consensus bacterial 6-phytase variant (PhyG) in broilers. Twelve treatments were tested with 10 replicate pens (30 birds/pen, day-old Cobb 500 mixed sex broilers). A completely randomized block design was used with 3x4 factorial arrangement comprising three basal diets (BD) with PP levels of 0.25% (BD1), 0.29% (BD2) and 0.33% (BD3), and four PhyG dose levels (0, 500, 1500, and 3000 FTU/kg). The basal diets were formulated with low dig P (0.232, 0.227, 0.187, and 0.170%); Ca (0.719, 0.621, 0.551, and 0.501%); dig Lys (1.153, 1.062, 0.974, and 0.894%) and ME (2928, 2978, 3031, and 3051 kcal/kg) in starter, grower, finisher 1 and 2, respectively, compared to commercial standard considering the contribution of the phytase. Diets were based on corn, wheat, soybean meal with rapeseed and sunflower meal and fed in crumble (starter) or pelleted form (other phases). Performance was measured in each phase and accumulative through 42d and mortality-corrected FCR was calculated. On day 42, tibias were collected from 4 birds/pen for (individual) breaking strength and (pooled) tibia ash. Data were analyzed as a 3x4 factorial analysis of variance using Minitab. Tukey's HSD was used for mean separation. Interaction between PP and phytase was observed for 42d BW, 1-42d feed intake and bone strength. In the absence of phytase, the diet with low PP (BD1) reduced these parameters vs. BD2 and BD3. In the presence of phytase, all treatments improved these parameters vs. BD1 without phytase and were not impacted by PP levels. Increasing phytase dose from 0 to 3000 FTU/kg increased 42d BW ($P<0.0001$) from 2592 to 3065g for 0.25% PP, from 2817 to 2996g for 0.29% PP, and from 2922 to 3056g for 0.33% PP. Increasing phytase dose from 0 to 3000 FTU/kg increased breaking strength ($P=0.024$) by 12.8, 10.1, and 8.4 kg force (kgF) in the 0.25, 0.29, and 0.33% PP, respectively. Across PP levels, overall 1-42d FCR was exponentially improved ($P<0.01$) with increasing phytase dose 0 (1.701), 500 (1.673), 1500 (1.638), and 3000 FTU/kg (1.630). Tibia ash was exponentially increased ($P<0.05$) with increasing phytase dose 0 (41.5%), 500 (43.0%), 1500 (44.3%), and 3000 FTU/kg (45.2%) across PP levels. In conclusion, the nutrients and energy deficiency in BD especially with low PP reduced performance and bone quality, PhyG compensated these reductions in basal diets, improved growth performance, and bone quality of broilers, regardless of PP levels. Increasing PhyG dose from 500 to 1500 or 3000 FTU/kg further improved ($P<0.05$) feed efficiency and this could lead to production benefit (PhyG at 1500 FTU/kg reduced feed cost/kg BWG by on average of 1.8% vs. 500 FTU/kg).

Key Words: broiler; performance; phytase; phytate

385P Effects of an exogenous protease in diets with three crude protein levels on broiler ileal digestibility. Lina Peñuela-Sierra^{1*}, Paula Lozano-Cruz¹, Jhon Mejia-Abaunza¹, V.L. Aragao- Neto¹, Muhammad Ali¹, Joaquin Cabanas-Ojeda¹, Maria Alfaro-Wisaquillo¹, Gustavo

Quintana-Ospina¹, Bindhu Vasanthakumari², Alexandra Wealleans², Edgar Oviedo- Rondón¹, ¹*North Carolina State University, Raleigh, North Carolina, United States*, ²*Kemin Industries, Des Moines, Iowa, United States*.

Low crude protein (CP) diets and exogenous protease have shown benefits in reducing costs and minimizing environmental impact. However, previous studies with low CP diets generally add crystalline amino acids (AA) with higher digestibility, and consequently, the effects of enzymes on ileal amino acid digestibility are affected. The objective of this experiment was to evaluate the impact of an exogenous protease on ileal amino acid digestibility of broiler grower diets with three crude protein levels. A total of 294 Ross 308 AP male broilers were placed in 42 battery cages. Up to 7 days of age, all chickens were fed one common starter diet in crumbles. Six treatments resulted from a 3 x 2 factorial arrangement of three crude protein levels (17, 19, and 21% CP) and two protease levels (0 or 300 g/ton), with seven replicates per treatment and seven chicks per replicate. Data were analyzed in a completely randomized design and mean separation with Tukey's test using JMP. Three isocaloric (3,100 kcal/kg ME) basal diets were formulated to reduce CP, but all diets maintained digestible Lys in 5.47% of CP and the same ideal protein profile. Crystalline Lys, Met, and Thr were used to obtain desired AA levels of balanced protein but not to keep similar AA concentrations across the three diets. The Lys:calorie ratios were 0.30, 0.34, and 0.37 %/Mcal ME. All diets contained titanium oxide as an indigestible marker. The exogenous protease or sand was added on top of these basal diets. On day 24, all birds were sacrificed with CO₂ to collect the digesta of the 2/3 distal portion of the ileum. Ileal contents were frozen at - 20 °C and were analyzed for DM, titanium, and AA acid content by HPLC. Diets were analyzed for protein, AA, titanium, and DM. The parameters determined were ileal DM, apparent AA, CP digestibility, and Lys retention. No interaction effects were observed ($P > 0.05$) in BW and BW gain. Interaction effects ($P < 0.05$) were observed in FCR and for almost all ileal AA digestibility coefficients, except for Cys and Trp. Live performance worsened as CP decreased. In this two AA, the main effect of protease was observed ($P < 0.05$). The protease improved the ileal digestibility of all AA only in diets with 21% CP. The protease improved the digestibility of Cys and Trp ($P < 0.05$) independently of CP level. No differences in ileal AA digestibility due to dietary CP level ($P > 0.05$) were detected, except for Cys digestibility ($P < 0.01$), which was better in 19% diets than in 21% CP, while 17% CP was intermediate. A similar balance of AA across all diets may cause similar ileal AA digestibility. The protease positively affected ileal digestibility when AA levels exceeded chicken requirements.

Key Words: broilers; exogenous protease; low crude protein; Amino acid digestibility

386P Effects of endogenous bacterial α -amylase in broiler diets on growth performance, intestinal morphometric characteristics, cecal short-chain fatty acid content, and blood metabolites. Ricardo Nunes^{1*}, Lidiane Datsch¹, Raquel Schoffen¹, Guilherme Luiz Tesser¹, Cleison De Souza¹, Andre Avila¹, Nilton Rohloff Junior¹, Cinthia Eyng¹, Claudia Bruch², Felipe Potenza Campos¹, Daniel Monteiro³, Thiago Ribeiro³, ¹*Unioeste, Marechal Cândido Rondon, Brazil*, ²*UTFPR, Dois Vizinhos, Brazil*, ³*Tectron, Toledo, Brazil*.

The use of enzymes in the formulation of animal diets is a practice that plays an important role for the industry. Carbohydrase can reduce digesta viscosity, improve nutrient digestibility, and performance of broilers. The objective of this study was to evaluate endogenous α -amylase inclusion in broiler diets and a metabolizable energy (ME) reduction of 100 kcal/kg. One-d-old chicks (n = 640; Cobb 500) with initial body weight of 47.11 ± 0.79 g were used in a completely randomized design experiment composed of 4 treatments and 8 replicate pens (20 birds per pen). Treatments were: one positive control (PC) with no ME reduction; negative control (NC) with a reduction of 100 kcal/kg ME; NC + 100 g/ton of α -amylase and, NC + 200 g/ton of α -amylase. Corn and soybean meal-based diets were formulated to meet the birds' nutritional requirements and broilers were fed from d 1 to 21, 22 to 35 and, 36 to 42 periods. Weight gain (WG), feed intake (FI), and feed conversion ratio (FCR) were determined on d 21 and 42. On d 35, a total of 32 broilers (one broiler per pen) were randomly selected and euthanized for duodenum and jejunum morphometric evaluations. Cecal content was solubilized with 1% sodium hydroxide solution and then the supernatant acidified with 50% ortho-phosphoric acid to quantify the concentration of short-chain fatty acids. On d 40, one broiler per pen (32 broilers total) was randomly selected and fasted for 6 h prior to blood collection. Serum samples were analyzed for α -amylase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine phosphokinase (CPK), lactate dehydrogenase (LDH) activity, and glucose (GLU) concentration using commercial kits with a high-performance automatic spectrophotometer. A unidirectional analysis of variance (ANOVA) was performed using the GLM proc at 5% significance. Positive control-fed birds presented a higher WG ($P < 0.0001$), and birds fed with the NC diet had higher FCR ($P = 0.0002$) compared to the other treatments. For the intestinal morphometric analysis, duodenal crypt-villus ratio was higher for treatments with ME reduction ($P < 0.0001$) and no effects were observed for jejunum (include p value). Enzymatic activity and glucose concentrations were similar among treatments demonstrating that there were no metabolic interferences in animals. Highest concentrations of acetate ($P = 0.0007$) and butyrate ($P = 0.0102$) were observed on animals fed with diets containing 200 g/ton of α -amylase, and the lowest concentrations were observed on NC-fed broilers. In conclusion, α -amylase utilization in

diets improved broiler FCR after 42 d. Also, cecum concentrations of acetate and butyrate were higher on birds fed with diets containing 200 g/ton of α -amylase.

Key Words: gut; starch; enzymes; carbohydrates ; short chain fatty acid

387P Effect of two NSP-enzymes on metabolizable energy levels of broilers from 19 to 24 day. Rodrigo Knop^{1*}, Nicolas Martinez¹, Adebayo Sokale¹, Luis Albino², Thaisa de Franca², Guilherme Pasquali³, ¹BASF, Florham Park, New Jersey, United States, ²Viçosa University-MG, Brasília, Brazil, ³BASF, Lampertheim, Germany.

Supplementation of corn soybean meal-based diets with non-starch polysaccharide (NSP) degrading enzymes may enhance energy utilization and growth performance of broiler chickens. However, there may be differences in the nutrient release by different NSP enzymes. This metabolic study evaluates the effect of 2 NSP-enzymes, a 1,4- β -xylanase + 1,4- β -glucanase produced by *Aspergillus niger* (Natugrain TS; ENZ 1) and 1,4- β -xylanase produced by *Trichoderma citrinoviride* (ENZ 2), on crude protein and energy metabolism of broiler. After a 14-day adaptation period on the floor, birds were moved to cages for a 5-day adaptation period and 5 days fecal collection. A total of 126 male Cobb broilers (6 birds \times 7 cages/treatment) were randomly allocated to 3 dietary treatments in a randomized complete block design. All diets were formulated with a reduction of 200 Kcal/kg energy level in comparison to the Rostagno, 2017 recommendation (3050 kcal/kg). The 3 experimental diets tested in this study were: Negative Control (NC) with 2850 Kcal/kg energy; NC + ENZ 1 (ENZ1) and NC + ENZ 2 (ENZ2). Both enzymes were supplemented based on manufacture's recommendation. The crude protein, apparent metabolize energy (AME), Nitrogen-corrected AME (AMEn) and crude protein retention on dry matter basis, were analyzed by One-way ANOVA. Treatment means were further separated using Tukey's HSD test. AME was significantly higher in ENZ1 compared to NC ($P = 0.025$) by 121 Kcal/kg, with ENZ2 as intermediate. AMEn was significantly higher in ENZ1 compared to the NC ($P = 0.037$) by 103 Kcal/kg, with ENZ2 as intermediate. Crude protein retention was significantly higher in ENZ1 compared to the NC ($P = 0.015$) by 6%, with ENZ2 as intermediate. In conclusion, the current study demonstrated that the addition of Natugrain TS, a β -xylanase + β -glucanase product at a standard dose increased feed energy and protein metabolism in broilers that were fed a reduced-energy diet.

Key Words: broilers; crude protein; energy; NSP enzymes

388P Effect of Novel Mannanase on growth performance of broilers from 1 to 42 days. Rodrigo Knop^{1*}, Nicolas Martinez¹, Adebayo Sokale¹, Anna Fickler², Luis Albino³, Thaisa de Franca³, ¹BASF, Florham Park, New Jersey, United States, ²BASF, Lampertheim, Germany, ³Viçosa University-MG, Brasília, Brazil.

Energy is one of the most expensive components of poultry diets. Enzymes can improve the digestibility of the feed and thus, serve as an option to lower the dietary energy level. This study evaluates the effect of reducing feed energy levels and the use of a novel endo-1,4- β -D-mannanase (Natupulse[®] TS), derived from *Trichoderma reesei* on growth performance parameters of broilers. A total of 900 one-day-old male Cobb broilers (30 birds \times 10 pens/treatment) were randomly allocated to 3 dietary treatments over 2 feeding phases, Phase I (d1 - 21) and Phase II (d21 - 42) in a randomized complete block design. A corn soybean meal-based diet was formulated in 3 treatments: Positive Control (PC) with 3050 and 3200 Kcal/kg levels in Phase I and II, respectively; Negative Control (NC) with 2850 and 3000 Kcal/kg in Phase I and II, respectively (reduction of PC diet by 200 Kcal/kg); NC + mannanase (800 TMU/kg of Natupulse[®] TS). Reduction in energy level between PC and NC diets was achieved by the reducing 22.7 kg soy oil per metric ton of feed. Feed intake (FI), body weight (BW), body weight gain (BWG) and feed conversion rate (FCR), were analyzed by One-way ANOVA according to Randomized Complete Block Design. Treatment means were further separated using Tukey's HSD test. At 21 days, BW ($P = 0.005$) and feed efficiency ($P = 0.001$) were significantly improved in the NC + mannanase treatment in comparison to the NC, and not statistically different from the PC. However, no statistical difference among treatments was observed for FI. At 42 days, FCR was significantly ($P = 0.001$) lower in the NC + mannanase treatment in comparison to the NC, and not significantly different from the PC. Furthermore, BW ($P = 0.043$), and FI ($P = 0.007$) were significantly higher in the NC + mannanase treatment in comparison to the NC with PC as intermediate. In conclusion, novel mannanase (Natupulse[®] TS) supplemented at the recommended dose level of 800 TMU/kg significantly improved the growth performance of broilers fed diets with 200 Kcal/kg energy reduction.

Key Words: performance; enzymes; mannanase;

389P The effects of a multicarbohydase and a mono-component xylanase on the growth performance and cecal short-chain fatty acid (SCFA) profile of broiler chickens. Kyle Smith^{1*}, Tri Duong¹, Niall Higgins¹, Brett Lumpkins², Bradley Turner³, Ronald Pegg⁴, Sara Llamas Moya¹, ¹Kerry, Woodsboro, Texas, United States, ²Southern Poultry Research, Inc., Athens, Georgia, United States, ³Poultry Research Partners, Athens, Georgia, United States, ⁴University of Georgia, Athens, Georgia, United States.

Exogenous non-starch polysaccharide enzymes remain at the forefront of the strategies available to enhance the nutritional value of poultry feeds. Mono-component xylanases are successful in facilitating the use of cereal hemicelluloses by broilers. However, poultry diets include a broader range of substrates that warrant use of effective multicarbohydases targeting cellulose and hemicellulose

from both cereals and oilcakes. In this study, the effect a multicarbohydase (MC) containing xyloglucanase and β -mannanase, and a mono-component xylanase (XY) were evaluated in broiler chickens. Male broiler chicks (Cobb, n=2,080) were allocated to four experimental treatment groups with 13 replicate floor pens with 40 birds each arranged as a randomized complete blocked design. Birds were fed an industry-type corn-soybean meal-DDGS diet (PC) or an equivalent diet with reduced energy density by 100 kcal/kg ME (NC), which was supplemented with either MC or XY. A 3-phase feeding program was provided, with water and mash feed offered *ad libitum*. Growth performance was evaluated by determination of BW, total feed consumption and FCR through 42 d of production. On d1 and d42 a representative litter sample was collected from each pen for pH, moisture, and ammonia analysis. On d42, cecal contents were collected and pooled from 5 birds/pen for short chain fatty acid (SCFA) analysis. All data was analyzed using ANOVA. Although final BW was not affected by treatment ($P > 0.1$), significant differences in total feed consumption indicated that birds on the NC diet consumed more feed throughout the study than the PC and MC group ($P = 0.039$). Reducing the energy density of the diet (NC) resulted in poorer FCR in comparison to the PC group ($P < 0.001$). Both MC and XY improved FCR, but only MC-treated birds showed efficiencies equivalent to the PC group. Litter characteristics were unaffected by treatment ($P > 0.1$). Ceca SCFA production, specifically propionic and n-valeric acid ($P < 0.05$), highlighted a 15% lower concentration of total SCFA in the MC group when compared to the NC treatment. Overall, these data suggest that MC was effective in improving the growth performance of birds fed a low energy dense diet, and it may have caused a positive shift in nutrient utilization towards the foregut, reducing the potential substrate available for fermentation in the hind gut.

Key Words: broilers; energy density; xyloglucanase; multicarbohydase; SCFA

390P A novel precision-fed rooster assay for determination of phytic acid disappearance in feedstuffs. Benjamin Parsons^{1*}, Pam Utterback², Jason Emmert², Samuel Rochell¹, Carl Parsons², ¹University of Arkansas, Fayetteville, Arkansas, United States, ²University of Illinois at Urbana-Champaign, Urbana, Illinois, United States.

The objective of this study was to develop a precision-fed rooster assay that is suitable for determination of phytic acid (myo-inositol 1,2,3,4,5,6-hexakis; InsP₆) disappearance in plant-based feed ingredients. A 48 h precision-fed rooster assay was used to measure InsP₆ disappearance using conventional White Leghorn roosters. A minimum of 4 individually-caged roosters per treatment were fasted for 26 h prior to being precision-fed (crop intubation) between 15 and 30 g of sample. After roosters were precision-fed, excreta (feces + urine) were quantitatively collected for 48

h. Soybean meal, soybean hulls, canola meal, conventional distillers dried grains with solubles (DDGS), palm kernel meal (PKM), and wheat bran were evaluated in Experiment 1, whereas wheat middlings and rice bran were evaluated without exogenous phytase and with 1,000 and 1,800 FTU/kg phytase in Experiment 2. Data from both experiments were subjected to a one-way ANOVA for a completely randomized design. Data from Experiment 2 were also subjected to two-way ANOVA for a 2 × 3 factorial arrangement of treatments with ingredient (wheat middlings or rice bran) and phytase level (0, 1,000, or 1,800 FTU/kg) as main effect variables. In Experiment 1, InsP₆ disappearance ranged from 3 to 95% among all ingredients. The InsP₆ disappearance for conventional DDGS (95%) was the highest ($P < 0.05$), wheat bran and soybean hulls were intermediate (47%), PKM was low (24%), and soybean meal and canola meal were very low (3 to 5%). The InsP₆ disappearance for wheat middlings and rice bran without exogenous phytase in Experiment 2 was 58% and 26% respectively, which was increased ($P < 0.05$) to 74% and 53% with the addition of 1,800 FTU/kg phytase, respectively. In Experiment 2, there was a significant ingredient × phytase interaction ($P < 0.05$). Thus, the inclusion of phytase at both 1,000 and 1,800 FTU/kg resulted in a significant improvement ($P < 0.05$) in InsP₆ disappearance for rice bran; however, only the addition of 1,800 FTU/kg phytase resulted in an increase in InsP₆ disappearance for wheat middlings. The InsP₆ disappearance for wheat middlings and rice bran without exogenous phytase in Experiment 2 was 58% and 26%, respectively, which was increased ($P < 0.05$) to 74% and 53% with the addition of 1,800 FTU/kg phytase, respectively. In conclusion, these results suggest the precision-fed rooster assay can be used to evaluate phytic acid disappearance in plant-based feed ingredients and to evaluate the effect of exogenous phytase on phytic acid disappearance.

Key Words: phytase; phytic acid; precision-fed rooster; feed ingredients

391P Effect of synbiotic on intestinal histomorphometry of quails, experimentally infected with field strain of *Salmonella gallinarum*. Asad Khan^{1*}, Asim Aslam², Muti Rehman², ¹Riphah College of Veterinary Sciences, Lahore, Pakistan, ²UVAS, Lahore, Pakistan.

This study was planned to investigate the effect of synbiotic on intestinal histomorphometry and growth rate of quails experimentally infected with field strain of *Salmonella gallinarum*. Day old Japanese quails (120), average body weight 6.09±1.1 g, were randomly assigned into 4 groups A, B, C and D. Quails of group A and B were given synbiotic on daily and weekly basis respectively, along with challenge while group C was negative control and group D was positive control group. A total 60 organ samples of intestine were collected from apparently healthy and recently euthanized quails respectively to isolate the field

strain of *Salmonella gallinarum*. Bacterial isolation and molecular identification were performed in accordance with laboratory diagnostic culture techniques and PCR. Results from current study indicate that non antibiotic feed additive such as synbiotic boosted the gut histomorphometric parameters including the villus height, villus width and crypts depth under the challenge of *Salmonella gallinarum*. There was significant increase in all these parameters due to synbiotic feeding except the negative control group which showed the lowest values. Statistical results of micrometry were analyzed by using analysis of variance (ANOVA), through SPSS 16.0 with Tukey's post HOC test and values with significant difference having p value < 0.05. At day 21, D achieved maximum villus height of 636.88 ± 65.93^b μ m in duodenal mucosa, whereas, maximum villus width of 182.01 ± 15.40^e μ m in duodenal mucosa was achieved by group A. Maximum villus height of 276.89 ± 21.16^b μ m and crypt depth of 26.66 ± 1.15^a μ m in jejunum mucosa were recorded in group A. FCR mean value in group D showed improved FCR (1.84) followed by group A (2.09). Group B showed an average FCR value (2.24) and group C has lowest FCR (2.36). There was significant increase in morphometric parameters of duodenal and jejunum mucosa in the groups fed synbiotic as compared to the negative control. This study supports the beneficial effects of synbiotic on intestinal health of Japanese quails by improving FCR.

Key Words: histomorphometry; Synbiotic; *Salmonella gallinarum*; Japanese quails

392P Not presented.

393P Standardized natural citrus extract as efficient as antibiotic growth promoters in enhancing broiler chicken growth performances: Case study in Peru. Sekhou Cisse^{1,2}, Mathilde Buffière¹, David Guilet^{2,3}, Mohammed el Amine Benarbia^{1*}, ¹Nor-Feed SAS, Angers, Maine et Loire, France, ²EA921 SONAS, Beaucouzé, Maine et Loire, France, ³FeedInTech, Beaucouzé, Maine et Loire, France.

Antibiotic growth promoters (AGPs) are widely used in order to improve animals' growth performances. However, the use of AGPs is positively correlated to an increase in antibiotic resistance phenomenon thus, compromising their use for therapeutic purposes both in animals and in humans. To overcome this, plant extracts such as Citrus extract are promising alternatives. The aim of this trial was to evaluate the use of a Standardized Natural Citrus Extract (SNCE) in an AGPs replacement strategy. 264 one-day-old Cobb-500 birds from a commercial farm in Peru were divided into 3 groups: A negative control group composed of broiler chickens fed with a standard diet without supplementation; A positive control group was fed with the same standard diet than the negative control group and supplemented with 1000 ppm of Baczin® (antibiotic), 150 ppm of Neomycin (antibiotic) and 500 ppm of Decoquinat (anticoccidial); A Citrus group in which Neomycin and Baczin were

replaced by 250 ppm of SNCE. Each group was composed of 6 replicates (pen) of 14 or 15 broilers chickens each. Zootechnical parameters (weight, feed intake, mortality) were recorded weekly until day 42 and feed conversion ratio (FCR) was calculated for each period. Statistical analysis was performed by one-way ANOVA test using R Studio software. The pen was used as statistical unit and statistical significance was considered at $P < 0.05$. At the end of the trial, no significant differences were observed in terms of live weight when antibiotics were replaced by SNCE in the broilers' diet (3029 g Vs 3076 g, $P > 0.05$). However, the live weight of birds from the negative control was lower than the live weight of birds from the Positive control (2942g vs 3029 g, $P < 0.05$) and Citrus groups (2942g vs 3076 g $P < 0.05$). No significant difference was observed in the FCR of broilers from the positive control group (1,62) and the Citrus group (1,58). Nevertheless, the FCR of broilers from the negative control group (1,66) was higher compared to the two other groups (1,62 (positive control) and 1,58 (Citrus group)). No difference was observed in terms of mortality. Results from this trial showed that SNCE has a similar effect on poultry growth performances to AGPs and, associated with good farming practices, could be an efficient solution to raise broiler chicken without AGPs and contribute to limiting the antibiotic resistance phenomenon.

Key Words: growth performances; Citrus extract; Chemical growth promoters replacement; standardized natural solution

394P The effect of orthophosphoric acid supplementation in drinking water on broiler performance. Zlatko Janjecic^{1*}, Helga Medic², Estella Prukner-Radovic³, ¹University of Zagreb Faculty of Agriculture, Zagreb, Croatia, ²University of Zagreb Faculty of Food Technology and Biotechnology, Zagreb, Croatia, ³European Federation World Poultry Science Association, Zagreb, Croatia.

High productivity and efficient food conversion as imperatives in modern livestock production can be achieved through the use of certain additives in animal nutrition. Previous research suggests that the use of acidifiers has practical value with a positive effect on health, body growth and food utilization, and thus on the economic justification of use, especially in salmonellosis infections, subclinical forms of coccidiosis, sudden changes in microclimate and nutrition and mucosal diseases. Acidifiers are used as an antidiarrheal alone or in combination with antibiotics, sulfonamides, chemotherapeutics or coccidiostats. Orthophosphoric acid is one of the few harmless, non-toxic acids, so it serves in the food industry and as a means of acidifying food and many soft drinks. The aim of this study was to determine the effect of orthophosphoric acid supplementation in the fattening of broiler chickens, i.e. to determine body weight, feed conversion and mortality of chickens that received 0.1 and 0.2% orthophosphoric acid

supplementation in drinking water and compare them with the production results of the control group of chickens. The experiment used 240 male one-day-old chickens (Ross 308) divided into three groups of 80 chickens, a control group of chickens (K) and two experimental groups P-1 and P-2. Each group had 4 repetitions of 20 chickens. Chickens were fed commercial feed mixtures, from 1 to 14 days with feed mixture starter 21% c.p., from 15 to 35 days' finisher I with 18% c.p. and from 36 to 42 days with feed mixture finisher II with 18% c.p. and without coccidiostats. Data collected were subjected to a one-way analysis of variance in a completely randomized design using SPSS version 23. The significant means were separated using Duncan's multiple range test at 5% level of significance. After fattening for 42 days, chickens of groups K, P-1 and P-2 achieved an average body weight of 2758.68; 2833.43 and 2720.81 g. The average conversions of the compound feed were 1.77, 1.70 and 1.78 kg/kg. Mortality at the end of fattening was 4.20; 1.67 and 2.50%. It can be concluded that the addition of orthophosphoric acid in a concentration of 0.1% can be recommended in the fattening of hybrid chickens as the production results proved to be statistically significantly better for all the variable ($p < 0.005$) compared to other groups in the study.

Key Words: broiler performance; drinking water; orthophosphoric acid

395P Antagonistic effects of microalgae and organic trace mineral supplementations on the incidence of bacterial chondronecrosis with osteomyelitis lameness in broiler chickens. Adnan Alrubaye¹, Amer Hasan¹, Andi Asnayanti^{1*}, Khawla Alharbi¹, Abdulkarim Shwani¹, Intisar Hassan¹, Sonali Lenaduwe Lokuge¹, Wesley Vaught¹, Kyle burks¹, Douglas Rhoads¹, Walter Bottje¹, ¹*University of Arkansas, Fayetteville, Arkansas, United States.*

Bacterial Chondronecrosis with Osteomyelitis (BCO) lameness was first reported in 1972 and has become a significant animal health and welfare issue in the broiler industry. BCO lameness results in hundreds of millions of dollars in lost revenue annually. *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus cecorum*, and *Staphylococcus hyicus* have been isolated from BCO lameness in commercial broiler farms, while *Staphylococcus agnetis* was the most prevalent species identified in our research farm. Typically, the bacteria colonize the growth plates of long bones and lead to skeleton impairment. The pathogens reach the growth plates through leakage from the respiratory tracks and the gastrointestinal tract to the bloodstream by compromising intestinal tight junctions. Enhancing gut integrity, bone strength, and bactericidal activity are essential strategies for preventing BCO lameness in broilers. Microalgae have gained much attention as a potential substitution feed in broilers due to their nutritional and bioactive ingredients. Microalgae contain omega-3-fatty acids and calcium which are vital for bone tissue development. In addition, we have

published the efficacy of the Availa ZMC—zinc, manganese, and cobalt enhancing intestinal barriers. We then hypothesized that pairing microalgae and Availa ZMC supplementation would confer a synergetic effect in reducing BCO lameness. To test this hypothesis, five treatment groups of chicks were created in a randomized block design. T1 was a challenged group using wire-flooring pens to be the source of the BCO infection. Dietary treatments in litter-flooring pens were T2 (negative control diet), T3 (Availa ZMC at 1500 mg/kg), T4 (5% microalgae), and T5 (microalgae with Availa ZMC). The cumulative lameness incidences per treatment were recorded including scoring of tibial and femoral lesions. The results demonstrated that supplementation of Availa ZMC significantly reduced lameness compared to the control diet ($P < 0.05$). Adding ZMC and microalgae gave lower lameness than the control feed and was significantly better than microalgae alone but was statistically higher lameness than Availa ZMC alone ($P < 0.05$). This result attested to the efficacy of Availa-ZMC in protecting the broiler chickens against BCO lameness. In contrast, microalgae supplementation resulted in a deleterious effect on the BCO lameness indicating enhanced translocation of pathogens into the blood, resulting in infection of the growth plates and eventually BCO lameness. The mixed microalgae and Availa-ZMC treatment group reduced the microalgae's negative effect, accentuating contradictory effects of microalgae and Availa-ZMC supplementations.

Key Words: Broiler; Lameness; Microalgae; Chondronecrosis; Organic trace mineral

396P Meta-analysis on the effect of protected sodium butyrate supplementation on performance and gut morphology of broiler chickens. Lisa Arnalot¹, Simon Fontaine^{1*}, William Lambert¹, Tristan Chalvon-Demersay¹, ¹*Metex Noovistago, Paris, France.*

Gut health issues in broiler chickens have been managed by the use of antibiotics thus increasing the risk of outbreak of antimicrobial resistant bacteria. In this context, butyric acid supplementation has received increased attention related to its beneficial effects on the gut and its potential ability to decrease the need for antibiotic use. Butyric acid is commonly supplemented as encapsulated forms to have a targeted release in the whole digestive tract including cecum and colon, thereby enhancing its efficiency. In this meta-analysis, we aggregated and analysed the data of the published literature on the effect of encapsulated sodium butyrate supplementation on performance and gut morphology in broiler chickens. Our systematic search on Pubmed and Google Scholar combining the following keywords: [chickens] OR [broilers] AND [Butyrate] OR [Butyric acid] AND [Protected] OR [Encapsulated] OR [Coated] AND [Performance] OR [Gut morphology] yielded 35 studies including 71 control and 103 supplemented groups and investigating the effect of protected sodium butyrate supplementation on mortality,

performance and/or gut morphology in broiler chickens. Data were expressed in percentage of the control treatments and analysed using a general linear model with the study as a fixed factor and the dose of supplementation as a covariable using Minitab software. Gut morphology parameters from different intestinal segments were aggregated. The interaction between the response to the supplementation and the presence of a challenge was also investigated. Body weight gain was significantly increased in response to the level of supplementation of protected sodium butyrate ($P = 0.003$). The supplementation tended to increase feed intake ($P = 0.064$) and to decrease feed conversion ratio ($P = 0.100$) and mortality ($P = 0.067$). Regarding gut morphology, the supplementation improved significantly villous height ($P = 0.014$) but did not affect crypt depth ($P > 0.05$) which resulted in a trend towards an increase of villous height to crypt depth ratio ($P = 0.056$). For body weight gain, feed conversion ratio, mortality, villous height, crypt depth, villous height to crypt depth ratio, there was an interaction between the supplementation and the presence of a challenge ($P < 0.05$). This study confirms the efficiency of the supplementation of protected sodium butyrate to improve performance and gut morphology in broiler chickens. Interestingly, for most parameters, there was an interaction between the supplementation and the presence of a challenge suggesting that broiler chickens benefit from the supplementation to a greater extent when they are challenged.

Key Words: Performance; Meta-analysis; Broiler; Butyrate; Gut morphology

397P Administration of a postbiotic mitigated effects of experimental subacute necrotic enteritis in broiler chickens. Tri Duong^{1*}, Roshan Adhikari¹, Ishab Poudel², Pratima Adhikari², Brett Lumpkins³, Sara Llamas Moya¹, ¹Kerry Group, Beloit, Wisconsin, United States, ²Mississippi State University, Mississippi State, Mississippi, United States, ³Southern Poultry Feed and Research, Inc., Athens, Georgia, United States.

Necrotic enteritis (NE) is a multifactorial disease 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 have increased the need to develop non-antibiotic interventions to mitigate the effects of NE in poultry production. Postbiotics, preparations of inanimate microorganisms and/or their components that confer a health benefit on the host, may be potentially important alternatives to antibiotics in managing NE because of their beneficial health effects. The aim of this study was to evaluate the effects of a postbiotic preparation of a proprietary probiotic Lactic Acid Bacterium in an experimental model of subacute NE in broiler chickens. Broilers were fed untreated feed (UNT) or feed supplemented with either an antibiotic (BMD) or postbiotic (POST), and subacute NE was induced experimentally

using a commercial *Eimeria* vaccine and *Clostridium perfringens*. Uninfected broilers fed untreated feed served as a positive control (PC). Broiler chicks ($n=2000$) were allocated to the four experimental treatments with 10 replicate pens of 50 birds arranged as a randomized complete block design. Growth performance was evaluated through 42 d of production, gross intestinal lesion scores were evaluated at 21 d post-hatch, and ileal histomorphology and relative expression of genes associated with inflammation and gut integrity were evaluated at 31 d post-hatch. All data were analyzed using one-way ANOVA. Treatment had no significant effect on BWG, ADG, or ADFI, overall. However, FCR was significantly affected by treatment ($P=0.006$), with PC-birds being more efficient than the UNT group. POST treatment reduced FCR to a level similar to the PC and BMD-treated broilers. A significant effect on the severity of gross intestinal lesions was observed ($P < 0.001$) with the UNT group having the most severe lesions when compared with the other treatment groups. A significant effect was also observed on villus height: crypt depth ratio (VH:CD) ($P=0.02$) with VH:CD being greater in the PC broilers as compared to the UNT and BMD treated broilers. VH:CD of the POST treated broilers was similar to the uninfected PC. Expression of CLDN-1 in the PC and POST treated broilers tended to be lower than the UNT and BMD treated broilers ($P=0.07$), suggesting treatment with postbiotic mitigated intestinal pathology of the subacute NE challenge. Thus, these data demonstrate administration of a postbiotic preparation from a probiotic Lactic Acid Bacterium may be used to mitigate the effects of subacute NE in broiler chickens.

Key Words: broiler; necrotic enteritis; postbiotic

398P Botanical extract improves gut structure, oxidative status, and performance in broiler chickens. Juan Javierre^{1*}, Mayra Diaz-Vargas², Cruz Enriquez², ¹Layn USA, Irvine, California, United States, ²UDCA, Bogotá, DC, Colombia.

The present study evaluated the effect of a botanical extract [TruGro MAX-(TM)] on growth performance, nutrient digestibility, tight junction proteins and antioxidant enzymes in broilers. A total of 360 one-day-old Cobb broiler chickens were allocated into three groups of eight replicates and 15 chickens per replicate. The animals were distributed in a completely randomized design with three treatments, antibiotic growth promoter negative (AGP-), antibiotic growth promoter positive (AGP+), and TM. All diets contained 1% CeliteR as acid insoluble ash (AIA) source for nutrient digestibility studies. Chickens were challenged with coccidia on day 5th of age, by spraying the litter of all pens with an oral coccidia vaccine (MSD Fortegra) at 10 times the regular dose. At 42d of age the animals were slaughtered to evaluate ileal nutrient digestibility and intestinal morphometry such as villus height, crypt depth, and V:C ratio. Additionally, the relative

abundance of proteins that make up the intestinal barrier such as Claudin 1, Claudin 5, ZO-1, and Occludin was analyzed using immunofluorescence. Liver antioxidant enzymes were analyzed using relevant kits. Nutrient digestibility was determined by proximal analysis of diets and ileal content, taking into account the correction factor provided by AIA in diet and ileal content. The results obtained were subject to one-way ANOVA, and Tukey test for pairwise comparisons using the R-project software. At the end of the experiment, the AGP- group had significantly ($p < 0.01$) lower body weight than AGP+ or TM. This can be explained by increased crude protein, ether extract and crude energy digestibility in BE and AGP+ diets, which were not significantly ($p < 0.05$) different among them, while significantly different ($p < 0.05$) to AGP- diet. Inclusion of TM in broiler diet improved intestinal morphometry parameters; increased expression of tight junction proteins Claudin-1, Claudin-5 and ZO-1; and increased SOD and GSH-Px in liver tissues. This indicates improved intestinal health, better nutrient absorption, and reduced oxidative stress, resulting in higher weight. We propose TM was able to equalize the effect of AGP based on increased expression of tight junction proteins and reduced oxidative status. This would have the effect of controlling the transfer on non-desirable intestinal molecules that may otherwise cause waste of energy and protein to fight inflammation and immunitary reactions. Likewise, a reduced oxidative status contributes to a lower chance of reactive oxygen species interfering with cell wall lipids and/or DNA replication.

Key Words: Performance; Intestinal barrier; Claudins; Occludin; Oxidative status

399P Effects of microalgae, probiotic, and phytogenic additives on growth performance, antioxidant enzyme activities, and intestinal immune response in broiler chickens. June Hyeok Yoon^{1*}, Su Hyun An¹, Sang Seok Joo², Myunghoo Kim², Changsu Kong¹, ¹Kyungpook National University, Sangju, Korea (the Republic of), ²Pusan National University, Miryang, Korea (the Republic of).

In broiler nutrition, microalgae and probiotic additives might have a beneficial effect on intestinal microflora which could improve growth performance and gut immunity. On the other hand, phytogenic additives have natural compounds that serve as a coccidiostat, antioxidant, or antimicrobial. The objective of this study was to evaluate the effect of various potential feed additives on growth performance, antioxidant enzyme activity, and intestinal immune response in broiler chickens. A total of 360 day-old Ross 308 male broilers were individually weighed and randomly assigned to six dietary treatments with six replicates in a randomized complete block design based on body weight (BW). Dietary treatments consisted of: 1) Control (CON; corn-soybean meal-based diet, without feed additive), 2) microalgae (CV; CON + 5 g/kg *Chlorella vulgaris*), 3) microalgae (TO; CON + 5 g/kg *Tetradesmus obliquus*), 4) probiotic (LP; CON + 1

g/kg *Lactocaseibacillus paracasei*), 5) phytogenic additive (PT; CON + 2 g/kg *Poncirus trifoliata* peel extract), 6) phytogenic additive (AA; CON + 1 g/kg *Artemisia argyi* extract). All birds were fed experimental diets from 1 to 10 days of age without any challenge. On day 9 post-hatch, BW and feed intake were recorded, and body weight gain and gain to feed ratio were calculated. On day 10, 3 birds with a median BW within a cage were selected from each replication (10 birds/cage) and asphyxiated by CO₂. Blood, jejunum tissue, and cecal digesta samples were collected. The profile of volatile fatty acids (VFA) in cecal digesta were analyzed by gas chromatography. An indirect ELISA was performed to quantify IgA in cecal digesta and antioxidant enzymes in serum. T-cell, B-cell, and macrophage populations in jejunum-lamina propria (LP) was measured by the flow cytometry analysis. Data were analyzed using the MIXED procedure of SAS and means were separated by Tukey's test. All feed additives did not affect growth performance. Compared to the AA group (0.017mM/g and 0.011mM/g), concentrations of isobutyrate (0.045mM/g) and valerate (0.022mM/g) increased ($P < 0.05$) in the LP group and CV group, respectively. All diets containing additives had no effects on the antioxidant enzyme activity in serum as well as cecal digesta IgA. An elevated percentage of CD3⁺CD4⁺ T-cell in jejunum-LP was observed ($P < 0.05$) in all diets containing additives compared with the CON diet. Whereas, lower proportion of CD3⁺CD8⁺ T-cells in the jejunum-LP was observed ($P < 0.05$) in a LP diet (56.5%) compared with the CON diet (70.5%). In conclusion, dietary feed additive supplementation in the broiler diet (1 to 10 days of age) showed changes in the cecal digesta VFA profile and the percentage of T-cells in the jejunum-LP.

Key Words: broiler; growth performance; feed additives; immunological response; plant extracts

400P Dietary late-cutting alfalfa directly affects the host and responses in broiler chickens vary between supplementation forms. Krysten Fries-Craft^{1*}, Stephan Schmitz-Esser¹, Elizabeth Bobeck¹, ¹Iowa State University, Ames, Iowa, United States.

Lipid soluble components in late cutting alfalfa are linked to beneficial immune and microbiome responses in murine models; however, poultry-specific outcomes are unknown. Coccidiosis due to *Eimeria spp.* results in performance reductions and economic losses which may be mitigated by 5th-cutting alfalfa. The study objective was to evaluate effects of 5th-cutting alfalfa hay and lipid-soluble hay extract on broiler performance, immunity, and the cecal microbiota during coccidiosis challenge. On d0, 432 Ross 708 broilers were placed in 24 floor pens (18 birds/pen) and randomly assigned to 1 of 3 dietary treatments (8 pens/treatment) for 42d, divided into 14d starter, grower, and finisher periods. Diets were individually formulated to be isocaloric/isonitrogenous and consisted of a control, 5% ground hay, and 0.25% lipid-soluble extract from 5th cutting

alfalfa. On d14, 4 birds/treatment were euthanized to collect blood and cecal contents. Half the remainder were orally inoculated with 10X Merck Coccivac®-B52 (Kenilworth, NJ), resulting in 6 total treatment groups. Body weight and FI were recorded weekly and tissue samples were collected at 3, 7, 14, and 21d post-inoculation (pi; 4 birds/ group). Immune cell populations in peripheral blood mononuclear cells were assessed by multicolor flow cytometry and cecal microbial communities profiled by 16S rRNA amplicon sequencing. Performance and immune cell data were analyzed using PROC MIXED (SAS 9.4) with diet, *Eimeria*, and diet×*Eimeria* fixed effects ($P \leq 0.05$). Microbial operational taxonomic unit (OTU) data were log-transformed and analyzed using PROC GLIMMIX with similar fixed effects. Prior to inoculation, birds fed alfalfa hay and extract diets ate 5.6-6.5% more and had 19.3-24.9% fewer peripheral CD3⁺ T cells than control-fed birds, but no differences were detected in the 100 most abundant OTUs. Regardless of diet type, the *Eimeria* main effect increased *Oscillospiraceae* relative abundance 1.7-fold and reduced grower FI and BWG by 9.7 and 15.3%, respectively, resulting in a 10-point less efficient FCR that was unresolved by diet ($P \leq 0.001$). In the first 3dpi, circulating Bu-1⁺ B cells increased 30.3% in challenged vs. unchallenged control-fed birds ($P = 0.004$), whereas a 59.6% increase in B cells was not observed until 7dpi in extract-fed challenged birds compared to unchallenged controls ($P = 0.0004$). In contrast, challenged hay-fed birds did not show Bu-1⁺ B cell changes throughout the challenge period. Collectively, these data indicate that alfalfa-associated immune modulation during *Eimeria* challenge varies between supplementation form and impacts the host directly but is not sufficient to protect performance in this model.

Key Words: Immunity; Microbiota; Broiler; Coccidiosis; Alfalfa

401P Effect of the probiotic *Escherichia coli* strain nissle on growth performance of broiler chickens. Samuel Nahashon^{1*}, Angela Settles¹, Thyneice Taylor-Bowden¹, ¹Tennessee State University, Nashville, Tennessee, United States.

Antibiotics have been used therapeutically as feed additive in poultry feeding and they have been reported to improve poultry performance. Consequently, their overuse has been associated with antimicrobial resistance in poultry and poultry products, which is transferable to the consumer, a risk to human health. Alternatives to antibiotics, such as the probiotic *Escherichia coli* strain nissle 1917 (EcN) have been evaluated for use in humans. There is, however, limited research on the potential use of EcN as a probiotic in poultry. The aim of this study was to assess the effect of EcN on growth performance of broiler chickens. In a completely randomized design, a total of 200 1-day-old broiler chicks were fed four dietary treatments comprising a standard corn-soy (SCS) control, SCS + soy protein, SCS + 10⁸ cfu/g EcN, and SCS + 10⁹ cfu/g EcN from hatch to 8

weeks of age (WOA). The EcN was encapsulated in soy protein, premixed in wheat middlings and homogeneously mixed in the SCS diets. Feed and water were provided *ad libitum*, birds were weighed weekly and body weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR) were determined weekly. Data were analyzed by one-way ANOVA using the General Linear Model of SAS and means were separated by the Least Square Means option. The birds fed diets containing 10⁹ cfu/g EcN exhibited mean BWG that were 15% higher than those of birds fed the control diets at 1-8 WOA ($P < 0.05$). The FC was higher and FCR was significantly lower ($P < 0.05$) in birds fed the 10⁹ cfu/g EcN. Broilers fed 10⁸ cfu/g EcN had improved BWG and FCR at 1-4 WOA ($P < 0.05$), however differences in these parameters among treatments were not significant at 5-8 WOA ($P > 0.05$). Therefore, feeding 10⁸ cfu/g EcN had slight improvement of BWG and FCR of broilers at 1-4 WOA, whereas feeding 10⁹ cfu/g EcN significantly increased BWG and reduced FCR of broiler chickens at 1-8 WOA.

Key Words: broiler chickens; growth performance; probiotics; feed conversion ratio; *Escherichia coli* strain nissle

402P Sustained probiotic supplementation promotes growth and performance in broiler chickens. Yuying Ren^{1*}, Mairui Gao¹, Ragini Reddyvari¹, Si Lu¹, Mary Anne Amalaradjou¹, ¹University of Connecticut, Storrs, Connecticut, United States.

Increasing concerns over antibiotic use in food animals and the emergence of antibiotic resistant pathogens led to the FDA directive curbing AGP use in poultry production. Thus, there is a critical need for an effective alternative to promote poultry health and performance. In this regard, in-feed probiotic supplementation serves as an effective alternative to AGPs. However, the period of embryonic development is critical to attaining optimum production efficiency. Therefore, in-ovo probiotic supplementation could be a potential alternative to promote growth and performance in broiler chicken. Towards this, our lab has demonstrated that early probiotic supplementation improved hatchability and hatchling quality in broiler chicks. As a follow-up, the present study investigated the effect of sustained probiotic supplementation on broiler performance. Beyond promoting hatchability and hatchling quality, sustained in-feed supplementation of *L. paracasei* DUP-13076 and *L. rhamnosus* NRRL- B-442 can further improve chick growth and performance. For the study, 312 one-day old Ross 308 chicks were randomly assigned to three groups (104 birds/group). The probiotic groups received a daily in-feed supplementation of either *L. paracasei* DUP-13076 or *L. rhamnosus* NRRL- B-442 (9 log CFU/g feed) for the entire duration of the study while the control received none. Broiler chicks were started on a 23% CP, 3000 kcal/kg ME ration and then placed on a 20% CP, 3200 kcal/kg grower/finisher ration at 3 weeks of age. Body weight and feed consumption were recorded during

the grow-out period. At the end of week 6, birds were sacrificed, and morphometric measurements were recorded. Further, breast muscle was also subject to meat quality analysis. The study was set out as a completely randomized design and data were analyzed using GraphPad (Version 9.3.1) with $p < 0.05$ considered as significantly different. Our results demonstrate that sustained probiotic supplementation significantly improved post-hatch performance in broilers ($p < 0.05$). Specifically, *L. rhamnosus* and *L. paracasei* supplementation improved body weight gain by 15-20%, RTE (ready to eat) weight by 18-23%, breast weight by 21-27% and leg weight by 15-17% when compared to the control. In addition, probiotics also significantly lowered FCR by 4-6%. Further, we did not observe any impact of probiotic supplementation on meat quality including color, pH and cook loss. Overall, sustained supplementation of LR and LP could be used to enhance the overall growth and performance in broiler chicken.

Key Words: broilers; Probiotic; sustained supplementation; growth promotion

403P Effects of supplementation of tannic acid on body composition in broilers and investigation of mechanisms: digestibility, enzyme activities, microbiome, and volatile fatty acid production. Janghan Choi^{1*}, Sudhir Yadav¹, Jinquan Wang¹, Benhemim Lorentz¹, Jefferson Lourenco¹, Todd Callaway¹, Woo Kim¹, ¹University of Georgia, Athens, Georgia, United States.

Supplementation of tannic acid (TA) can show either positive or negative effects on growth of broilers, the outcome depends on the dosage. The present study was conducted to investigate the effects of TA supplementation on protein and fat digestion, microbiota, volatile fatty acid (VFA) production, and body composition of broilers. A total of 320 male Cobb 500 broilers were randomly distributed to 4 treatments with 8 replicates including 1) tannic acid 0 (TA0): basal diet without TA; 2) tannic acid 0.5 (TA0.5): basal diet with 0.5 g/kg TA; 3) tannic acid 1.5 (TA1.5); and 4) tannic acid 2.5 (TA2.5). Orthogonal polynomial contrasts were used to evaluate the significance of linear or quadratic effects of different dosages of TA, and the segmented linear regression analysis was used to calculate the initiative dosage of TA for each parameter in broilers. Supplementation of TA linearly decreased apparent ileal digestibility (AID) of crude protein ($P < 0.01$). The AID of EE tended to be reduced at levels less than 525 mg/kg ($P = 0.08$). The lipase activities in the mid-jejunum tended to be reduced due to supplemental TA at levels greater than 595.3 mg/kg ($P = 0.09$). Supplemental TA tended to increase the relative abundance of the phylum Bacteroidetes in the cecal microbial communities ($P = 0.1$). Total cecal VFA production tended to be linearly reduced due to supplemental TA at levels greater than 850.9 mg/kg ($P = 0.07$). Lean weight tended to be reduced due to supplemental TA ($P = 0.07$). Fat weight was linearly decreased due to supplementation of TA in broilers ($P <$

0.05), and the ratio of lean to fat was linearly increased due to the supplemental TA ($P < 0.01$). Therefore, decreased lipase activities, fat digestibility, and VFA production resulted in reduced fat accumulation in broilers. This study indicates that TA supplementation had more impact on the fat accumulation rather than protein accumulation in broilers.

Key Words: microbiota; DEXA; Tannic acid; fat accumulation; volatile fatty acids

404P Effect of a proprietary essential oil product on growth performance, intestinal lesions, and oocyst counts of Cobb 500 broilers during a mixed *Eimeria* challenge infection. Kristjan Bregendahl^{1*}, Greg Mathis², Brett Lumpkins², Shameer Rasheed¹, Kristy Dorton¹, Mahmoud Masadeh¹, Mark LaVorgna¹, ¹Devenish Nutrition, Fairmont, Minnesota, United States, ²Southern Poultry Feed and Research, Athens, Georgia, United States.

Two experiments were conducted at Southern Poultry Feed and Research, Inc. (Athens, GA) to evaluate an essential-oil product (EO; DeviSTAT Broiler NA, Devenish, Fairmont, MN) on growth performance, intestinal lesions, and excreta oocyst counts (OPG) after a challenge infection with *Eimeria* coccidia. In each experiment, 1-d-old Cobb 500 broiler chicks (N = 144; as hatched) were allocated to Petersime battery cages in a randomized complete block design with 6 blocks and 8 chicks per cage, and kept for 20 d. The 3 treatments were uninfected–unsupplemented positive control (PC), infected–unsupplemented negative control (NC), and NC+EO. All birds were fed corn-soy based mash diets, and 0.5 g/kg EO was fed from d 1. On d 14, all birds except for PC were orally gavaged with 1 mL inoculum containing 100,000, 50,000, and 75,000 sporulated oocysts of *E. acervulina*, *E. maxima*, and *E. tenella*, respectively; PC birds received 1 mL saline. On d 20, intestinal lesion scores (ranging from 0 = none to 5 = severe) were evaluated from 5 randomly selected birds per cage, and oocysts counted in excreta from each cage using the floatation method. Feed intake and body weight were measured on d 0, 14, and 20. Data were analyzed by ANOVA and means separated with Fisher's least significant difference at $P \leq 0.05$. In Exp. 1, no significant difference was observed in body weight on d 20 (0.55, 0.46, and 0.47 kg for PC, NC, and NC+EO, respectively), but, in Exp. 2, the body weight on d 20 of NC+EO broilers was improved ($P = 0.002$) compared with NC (0.36 vs. 0.32 kg, respectively), both inferior to PC (0.43 kg). Broilers fed NC+EO had better ($P < 0.001$) feed conversion ratio than NC in both Exp. 1 (1.873 vs. 2.274) and in Exp. 2 (2.083 vs. 2.433) after the challenge (d 14 to 20). The corresponding feed conversion of PC (1.371 and 1.542 in Exp. 1 and 2, respectively) was superior to other treatments ($P < 0.001$) in both experiments. Feeding EO lowered ($P < 0.001$) lesion scores caused by *E. acervulina* from 2.8 to 2.3 and from 3.0 to 2.1; by *E. maxima* from 2.0 to 1.3 and from 1.6 to 1.3; and by *E. tenella* from 2.8 to 1.4 and from 2.6 to 1.2 in Exp.

1 and 2, respectively. Lesion scores were 0 for all *Eimeria* spp. for PC in Exp 1 and 2, significantly different from other treatments. In Exp 1., dietary EO lowered OPG from 245,000 to 90,000 oocysts per gram ($P < 0.001$) in challenged birds, whereas only a numerical difference (118,600 vs. 67,000; $P = 0.21$) was observed in Exp. 2. The OPG were 0 for PC in both experiments, significantly different from other treatments. Overall, the results show that supplementation of EO in broiler diets improved FCR and minimized lesions and excreta oocyst counts during an *Eimeria* infection.

Key Words: growth performance; *Eimeria*; lesion scores ; Broiler; Essential oil

405P Effect of *Bacillus amyloliquefaciens* CECT 5940 on growth performance, intestinal histomorphometry, and SCFA concentrations in broilers under *Eimeria* and *Clostridium perfringens* induced-challenge. Ricardo Nunes^{1*}, Nilton Rohloff Junior¹, Edevaldo Ianchinski^{2 1}, Cristine Kaufmann¹, Maisa de Carvalho^{2 1}, Ana Paula Costa¹, Clauber Polese¹, Cinthia Eyng¹, Rafaela Berto¹, Victor Naranjo³, Vinicius Teixeira³, Nerilson Nerilo³, ¹Unioeste, Marechal Cândido Rondon, Brazil, ²UTFPR, Dois Vizinhos, Brazil, ³Evonik Brasil LTDA, São Paulo, Brazil.

The objective of this study was to determine the effects of a probiotic (*Bacillus amyloliquefaciens* CECT 5940; Ecobiol®) supplementation compared to an antibiotic growth promoter (AGP) on growth performance, intestinal histomorphometry and short chain fatty acid (SCFA) concentrations in broilers subjected to coccidiosis plus *Clostridium perfringens* induced-challenge. A total of 726 day-old male Cobb 500 (initial BW = 43.44 ± 0.53 g) were distributed to 3 dietary treatments with 11 replicates pens of 22 birds per pen in a completely randomized design. The experimental diets included: 1) negative control (NC) without AGP, 2) positive control (PC) with 100 g/MT of 8% Enramycin, and 3) NC supplemented with 0.10% (1.0×10^6 CFU/g of feed) of Ecobiol®. Experimental diets in mash form were corn-SBM based and fed from 1 to 21 and 22 to 35 d of age. To induce necrotic enteritis infection, all birds were inoculated by gavage at 4 d of age with 20 times the dose of a coccidiosis vaccine (0.6 ml/bird of Biococcivet R®) and at 7 and 10 d of age with 5×10^7 CFU/bird of a field strain of *Clostridium perfringens* inoculum related to cases of necrotic enteritis. Growth performance parameters were determined for each feeding phase and the overall period and the concentrations of SCFA and intestinal histomorphometry at 35 d of age in 11 birds per experimental diet. As a statistical procedure, unidirectional analysis of variance (ANOVA) was performed using the GLM proc at 5% significance. Overall, broilers fed the NC diet had the lowest feed intake ($P < 0.001$), BW gain ($P < 0.01$) and poorest FCR ($P < 0.001$) compared to the PC and PB diets. However, broilers fed the PB diet had significantly higher feed intake, BW gain and improved FCR compared to birds fed the PC diet. The cecal concentrations of acetic

acid ($P = 0.698$) and propionic acid ($P = 0.860$) were similar between treatments; however, the cecal concentration of butyric acid ($P = 0.038$) was greater in broilers fed PB compared to the NC and PC diets. Birds fed the PB diet had greater crypt depth ($P < 0.001$) in jejunum compared to the NC and PC birds. Villus height ($P < 0.001$) and absorption area ($P < 0.001$) in jejunum were lower for broilers fed the NC diet but similar between broilers fed the PC and PB diets. These results demonstrate that supplementation with Ecobiol® improves growth performance of broilers compared to Enramycin with similar intestinal morphological characteristics while favoring a greater production of butyric acid by the intestinal microbiota. Therefore, Ecobiol® at a dose of 1.0×10^6 CFU g of feed was able to effectively replace the use of 100 g/MT of 8% Enramycin under a standardized induced intestinal challenge.

Key Words: broiler; performance; short chain fatty acid; AGP replacement; GI tract health

406P Novel isolates of *Bacillus Subtilis* improved FCR and changed cecum microbiota in broilers challenged with *Clostridium Perfringens*. Shweta Ghimire^{1*}, Xinwen Zhang¹, Jinglin Zhang¹, Changqing Wu¹, ¹University of Delaware, Newark, Delaware, United States.

Clostridium perfringens (CP)-induced necrotic enteritis (NE) causes high death and low growth performance in poultry. Globally, about US\$5 billion economic loss is caused annually by this disease. FDA has implemented an antibiotic phase-out plans in poultry feeds due to concern of antibiotic resistance rising demands for antibiotic substitutes. In this study, we will determine if two new *Bacillus subtilis* isolates, 103a and 62a, can control NE, improve feed conversion ratio (FCR), and cecum microbiota in broiler chickens. A 2-factor [Infection ($10^* \text{IBDV vaccine} + 10^8 \text{ cfu/bird CP vs no IBDV} + \text{no CP}$) \times probiotics (control (no probiotics), 103a, 62a, and mixed(103a+62a))] completely randomized feeding trial design was conducted for 28 days starting with day 1 male broiler chicks. Probiotic was given via water at 10^7 cfu/ml dose for 28 days. There were 15 birds in control and 5 in other treatment groups. Body weight and FCR were recorded at days 1, 10, 15, 20, 22, 24, 26, and 28, and mortality was observed each day. Organ weight, plasma sample and intestine NE lesion score were recorded at day 28. FCR, organ weight and plasma oxygen radical absorbance capacity (ORAC) were analyzed using two-way ANOVA. Cecum microbiota samples were prepared using Shoreline Biome microbiome assay Kit and demultiplexed files were generated by Sbanalyzer. These files were further analyzed in R using DADA2, taxonomy was assigned using silva-train-set file, and α -diversity indices were explored. Antimicrobial assay showed that 103a and 62a isolates inhibited CP. The chickens in infected mixed or 62a had lower FCR than non-infected counterparts. Significant decrease ($p < 0.05$) in FCR was detected in the infected 62a

group compared to the non-infected 62a group. The plasma antioxidant level in the infected 103a group was significantly lower than the level in the infected control group. Infected control group had higher small intestine NE lesion scores (mean= 2.66, SD=0.57) compared to other infected and noninfected groups. No significant difference was observed in organ weight in all infection*probiotics groups. Infected and non-infected control groups had higher mortality (4 in each group) compared to 103a, 62a and mixed with 2,1, and 0 mortality respectively. Alpha diversity of cecum microbiota showed that Shannon index was higher in infected group compared to that of non-infected group except for a decrease in 62a. Control infected group have significantly higher species abundant (Chao1) than non-infected 62a,103a, mixed, and infected 103a group. The promising impacts of probiotic isolates on chicken growth performance and NE control will be evaluated in a large feeding trial in future.

Key Words: FCR; Broilers; Microbiota; Necrotic Enteritis; *Bacillus Subtilis* 103a and 62a

407P Not presented.

408P Effect of the replacement of vitamin E by a blend of polyphenols in the diet on growth performance, and antioxidant status of the breast meat in broilers. N. Corrales¹, D. Menoyo¹, D. Baruch¹, P. Quaglia², Gonzalo Mateos^{1*}, ¹*Universidad Politécnica de Madrid, Madrid, Spain*, ²*Silvateam S.p.A, San Michele Mondovì, Italy*.

Vitamin E is an essential nutrient and an antioxidant used in practical diets for poultry to protect the metabolic processes of cell systems. Silvafeed[®] ATX, a blend of polyphenols (mainly tannins), is a phytogenic additive with antioxidant properties. This study aims to investigate the effects of the replacement of vitamin E by Silvafeed[®] ATX on broiler performance, and breast lipid oxidation. There were 5 diets with the same ingredient composition that varied only in the level of supplemented vitamin E (0 to 40 ppm with differences of 10 ppm among diets) and Silvafeed[®] ATX (300 to 0 ppm with differences of 75 ppm among diets). The experimental diets, fed as pellets, provided 10 ppm of vitamin E, and were based on corn and soybean meal. The feeding program consisted of two phases: starter (0 to 21 d) and finisher (22 to 38 d) and each diet was replicated 7 times (a floor pen with 16 straight run chicks). Growth performance was determined at 14, 21, and 38 d of age. Data were analysed, by phase and cumulative, as a CRD, using the MIXED procedure of SAS. When significant differences were detected, the Tukey test was used to separate treatment means. In addition, the effects of vitamin E and tannins on the different variables studied, were partitioned into their linear and quadratic components. From 0 to 38 d of age, no differences among treatments were detected for any of the variables studied. However, from 0 to 14 d of age, FCR improved linearly ($P < 0.001$) as the tannin content in the diet increased, indicating that, under

the conditions of the current experiment, the birds did not need any extra supplementation of the diet with vitamin E. At the end of the experiment, breast lipid oxidation was determined at 1, 3, and 7 days of storage at 4°C, using the 2-thiobarbituric acid method. Malonaldehyde concentration (MDA) increased significantly ($P < 0.001$) with storage time. However, the rate of increase varied depending on the vitamin E and Silvafeed[®] ATX combinations. At 1 d of storage, MDA were similar for all treatments but at 3 d, MDA tended to increase as the level of vitamin E decreased ($P = 0.074$). At 7 d of storage, MDA were higher in the diet that included 225 ppm of Silvafeed[®] ATX and 10 ppm of vitamin E and the negative control diet that did not include any supplemental vitamin E ($P < 0.01$). It is concluded that, under our experimental conditions, a corn-SBM diet supplemented with 20 or 30 ppm of vitamin E, results in similar growth performance and lipid stability than a diet supplemented with 40 ppm of vitamin E, provided that Silvafeed[®] ATX is included.

Key Words: broilers; growth performance; polyphenols; vitamin E; tannins

409P Effect of the replacement of dietary vitamin E by an extract rich in hydroxytyrosol, from olive mill wastewater, on growth performance, antioxidant status of the breast meat, and liver traits in broilers. N. Corrales¹, Gonzalo Mateos^{1*}, G. Talegón¹, F. Sevillano¹, D. Menoyo¹, ¹*Universidad Politécnica de Madrid, Madrid, Spain*.

Vitamin E places a role as an essential nutrient and an antioxidant to protect the metabolic processes of the cell systems in practical diets for poultry. The hypothesis of this experiment was that an extract based on a blend of polyphenols (14.1 g of hydroxytyrosol per kg of fresh product; Nutrición y Gestión S.L., Badajoz, Spain) from olive mill wastewater, could replace vitamin E as an antioxidant but not as a source of vitamin. There were 5 diets with the same ingredient composition that varied only in the level of supplemented vitamin E (0 to 40 ppm with differences of 10 ppm among diets) and hydroxytyrosol (30 to 0 ppm with differences of 7.5 ppm among diets). The experimental diets provided 10 ppm of vitamin E, and were based on corn and soybean meal and fed as pellets. The feeding program consisted of two phases: starter (0 to 21 d) and finisher (22 to 39 d) and each diet was replicated 7 times (a floor pen with 16 straight run chicks). Growth performance was determined at 7, 21, and 39 d of age. At the end of the experiment, breast lipid oxidation was determined at 1, 3 and 7 days of storage at 4°C, using the 2-thiobarbituric acid method. In addition, the weight and color of the liver were measured. Data were analysed as a CRD, using the MIXED procedure of SAS. From 0 to 7 d of age, FCR improved linearly ($P < 0.05$) as the hydroxytyrosol content in the diet increased. However, no differences in growth performance were detected after this age or cumulatively. The data indicate that under the conditions of

the current experiment, the birds did not need any extra supplementation in vitamin E. Malonaldehyde (MDA) concentration in the breast increased significantly ($P < 0.001$) with storage time. However, the rate of increase varied depending on the vitamin E and hydroxytyrosol combinations. The concentration of MDA in the breast, one day after storage, did not differ among treatments. After 3 and 7 d of storage concentrations were significantly higher ($P < 0.001$) in the breast of birds fed the negative control diet (0 ppm of added vitamin E). Yellowness of the liver was lower in the diet that included 7.5 ppm of hydroxytyrosol and 30 ppm of vitamin E ($P < 0.05$). However, no differences among treatments were detected for liver weight. It is concluded that, under our experimental conditions, combinations of 22.5 ppm of hydroxytyrosol and 10 ppm of vitamin E equalizes to a diet supplemented with 40 ppm of vitamin E in terms of performance and meat lipid oxidation.

Key Words: broilers; growth performance; polyphenols; vitamin E; hydroxytyrosol

410P Unraveling the potential effects of brown seaweed products on growth performance, blood biochemistry, immune response, and antioxidant capacity of broiler chickens challenged with heat stress. Fisayo Akinyemi¹*, Deborah Adewole¹, ¹Dalhousie University, Truro, Nova Scotia, Canada.

Brown seaweed (*Ascophyllum nodosum*) is known for its excellent antioxidant ability, but its impact on the growth performance and health of broiler chickens challenged with heat stress (HS) has not been fully elucidated. Given the potential benefits of seaweed, the current study aimed to determine the efficacy of brown seaweed meal (SWM) and extract (SWE) on growth performance, blood biochemistry, immunoglobulins (IgG and IgM), and antioxidant capacity of broiler chickens challenged with HS. A total of 336 one-day-old broiler chicks were divided equally into two rooms: 1) Thermoneutral room (TN; 24°C \pm 1 on d 21-27) and 2) Heat stress room (HS; HS; 32-34°C for 8 hr./day on d 21-27). In each room, birds were randomly allotted to four dietary treatments: 1) Negative control (NC; corn-wheat-soybean based diet), 2) NC + 1 ml SWE in drinking water, 3) NC + 2 ml SWE in drinking water, and 4) NC + 2% SWM in feed. Each treatment consisted of six replicate cages and each cage housed 7 birds. Rectal temperature (RT) of two birds per replicate was measured using digital thermometer to confirm induced HS on day 27. On day 28, two male birds/cage were euthanized for measuring blood biochemical, antioxidant, and immune status analysis. Data were analyzed as a 4 \times 2 factorial analysis of variance using GLM of Minitab LLC, (2019) software. 2% SWM inclusion significantly increased ($P < 0.05$) the body weight gain at the early phase (week 1), late phase (week 4), and at the end of the trial period. HS significantly reduced ($P < 0.05$) feed intake compared to TN birds. However, the body weight gain of heat-stressed birds was

not different from the TN birds. HS furthered increased ($P < 0.05$) the bird's RT, plasma concentrations of sodium, chloride, glucose, amylase, and uric acid but it reduced urea and creatine kinase concentrations. Heat stress increased ($P < 0.05$) IgM and IgG concentrations compared to TN birds, while the activity of superoxide dismutase was not affected by HS and dietary treatments. There was a significant interaction ($P < 0.05$) between dietary treatments and HS on plasma lipase and alanine aminotransferase (ALT). Lipase concentration reduced in HS birds fed with 2% SWM compared to other treatments. The plasma activity of ALT decreased in HS broilers that received 1ml SWE in drinking water compared to those fed with 2% SWM. Brown seaweed treatment and HS did not affect the relative weight of immune organs (bursa and spleen). Our results indicate that HS reduced feed intake and affected blood biochemical parameters and immunoglobulins, but dietary supplementation of brown seaweed products potentially mitigated the negative effects of HS by improving the plasma enzyme activities of heat-stressed birds.

Key Words: broiler chickens; growth performance; heat stress; Brown seaweed; immunoglobulins

411P Dietary inclusion of eMAX (*Bacillus* spp. and exogenous enzymes) improves the performance of broilers fed hypo-energetic diets without growth promoters. Claudia Castaneda¹*, Yordan Martinez², ¹Engrain LLC., Manhattan, Kansas, United States, ²Zamorano University, San Antonio de Oriente, Valle del Yeguaré, Honduras.

eMAX is a probiotic and enzymatic blend used as a natural growth promoter in broiler and layer diets to improve gut health and nutrient utilization without subtherapeutic antibiotics. The aim of this study was to evaluate the effect of dietary inclusion of eMAX on growth performance, carcass traits and economic impact of broilers. A total of 2240 one-day-old, unsexed broilers from Cobb 500 MV \times Cobb 500 FF genotype were analyzed for 35 days, following a completely randomized design with four dietary treatments, 12 replicates, and 57 birds per replicate. Dietary treatments consisted of a basal diet (BD) with corn-soymeal, an antibiotic growth promoter (AGP) and a commercial enzyme complex (EzC), D2: Corn-soy diet without AGP, EzC, coccidiostat, and with eMAX; D3: Corn-soy diet without AGP and EzC, with eMAX and 85 kcal/kg less ME in grower and in the finisher stage; D4: Corn-soy diet without AGP and EzC, with eMAX and 85 kcal/kg less ME in the grower stage and 140 kcal/kg less ME in the finisher stage. Three-phase diets including starter (d 0-9), grower (d10-18) and finisher (d19-35) were provided to the broilers. Data were analyzed via a one-way ANOVA with significant differences determined at $P \leq 0.05$ and separated using Duncan's test. Broilers fed diet 4 with the highest energy reduction and eMAX had a lower weight gain (WG) compared to the control ($P=0.001$). However, chickens fed

diet 3 with eMAX and a 3% energy reduction in the grower and finisher stage had a similar WG and FCR compared to the control ($P=0.081$). Birds fed diet 2 did not show an improved WG at normal ME levels. Broiler livability was higher than 98.5% for all the evaluated treatments. Carcass weight (CW) was not different between the basal diet and diet 3 and 4 ($P=0.08$). Likewise, dietary treatments did not statistically change the relative weight of the small intestine, spleen, and cecum ($P=0.641$, 0.174 , $P=0.381$, respectively). Diets with eMAX and lower ME resulted in lower cost per MT of feed and lower cost/kg of CW for diet 3 and 4 compared to the control diet. The results from this study indicate that the inclusion of eMAX in the diet having a lower ME, no AGP and no EZC did not affect performance and carcass weight, which also indicated a positive economic impact. Bacillus-based probiotic supplements such as eMAX could be a nutritional tool to reduce dietary energy levels, complementing their known beneficial effects in gut health.

Key Words: Probiotics; Broiler; Bacillus; No Antibiotics ; Economic impact

412P Growth performance and nutrient digestibility during the rearing phase in layer hens being fed diets supplemented with a bacterial origin xylanase. Mariana Lemos de Moraes^{1*}, Marcia de Souza Vieira¹, Heitor Rios¹, Boudry Christelle², Raquel Sanz García², Ludovic Lahaye¹, Elizabeth Santin¹, ¹*Jefo, Porto Alegre, Brazil*, ²*Belfeed, Dilbeek, Belgium*.

Bacterial xylanase presents high affinity to insoluble arabinoxylans and therefore offers novel nutrition strategies to degrade arabinoxylans on corn-soy-based diets. A study was conducted to evaluate the effects of a bacterial Xylanase from *Bacillus subtilis* supplemented in layer hen corn-soy-based diets during rearing phase on growth performance and nutrient digestibility. A total of 600 six-week-old Bovans White layer hens were randomly allocated in 60 cages, distributed in three treatments with 20 replicates and 10 animals/replicate until they completed 16w. Animals were fed experimental corn-soy-based diets formulated as follows: T1: Control (formulated to meet birds' nutritional requirements), T2: Control with reduction of apparent metabolizable energy (AME), and T3: Control with AME reduction + *Bacillus subtilis* xylanase at 100 g/t. A two-feeding program was used: grower (from 6w to 10w) and developer (from 11w to 16w). AME reductions were calculated using a dynamic matrix based on the arabinoxylans content of diets, being 91.9 kcal/kg in grower diets and 100 kcal/kg in developer diets. Feed intake (FI), body weight gain (BWG) and feed conversion ratio corrected for weight of dead birds (FCR) were evaluated weekly. At 10w, total excreta was collected to evaluate the total-tract retention of dry matter (DM), crude protein (CP), ether extract (EE), and apparent metabolizable energy (AME). Data were subjected to one-way ANOVA and, when significant, means were compared by Fisher test at a

level of 5% of significance (XLSTAT software). Layer hens fed diets with energy reduction with the addition of bacterial xylanase presented BWG similar to Control ($P > 0.05$) and 3.2% higher than layer hens fed energy reduction diets with no xylanase supplementation ($P < 0.05$). The highest FCR was observed for the treatment with energy reduction and no bacterial xylanase supplementation, being 2.4% higher than Control and 3.0% higher than the treatment with bacterial xylanase supplementation. No differences between Control and the treatment with xylanase supplementation was observed for FCR. The inclusion of bacterial xylanase on layer hen diets increased AME utilization and EE digestibility when compared to treatment with energy reduction and no xylanase supplementation by 103 kcal (3,158 kcal/kg vs 3,041 kcal/kg) and 3.8% (86.3% vs 83.1%) respectively, although no differences were observed for CP and DM digestibility between these treatments ($P > 0.05$). In conclusion, the supplementation of a bacterial xylanase on corn-soy-based diets with reduced AME level improved both growth performance and nutrient digestibility in layer hens during the rearing period.

Key Words: Nutrient digestibility; Laying hen; Energy utilization; Bacterial xylanase; Growth performance

413P Impact of water acidifiers type and dietary calcium and phosphorus on growth performance and bone mineralization in broilers. Angelica Solano García¹, Angel Alfonso-Avila^{1*}, Daniel Venne², Agnes Narcy³, Marie-Pierre Létourneau Montminy¹, ¹*Université Laval, Quebec, Quebec, Canada*, ²*Couvoir Scott, Scott, Quebec, Canada*, ³*INRAE, Nouzilly, France*.

Acidification of drinking water is use as an alternative to growth-promoting antibiotics. Antimicrobial properties of certain acids, their ability to lower the pH of drinking water and the buffering capacity of feed are linked with a beneficial effect on the physiology of gastrointestinal tract. The objective of this study was to test the impact of different type of water acidifiers: 1) no acidification, 2) Inorganic acidifier (INORGACID; phosphoric acid) and 3) organic acidifiers (ORGACID; mix) in a normal (NCaP) or in a sub-limiting Ca and P diet (LowCaP; -15% Ca and P). Level of inclusion of acidifying agents varied according to the target pH of the water (5.8). A total of 2700 one-day-old male broilers (Ross 308) were randomly assigned to the 6 treatments, with 10 replicates of 45 chicks per replicate for 3 growing phases (0-10, 11-21 and 22-30 days). Growth performances were determined in each phase. Water consumption was measured daily by pen. On day 10, 21, and 30, a blood sample was collected on 1 bird per pen ($n=60$) before slaughter for DXA scan measurements to obtain bone mineral content (BMC) as well as fat and lean mass. Results showed a decrease in average daily gain (ADG; -5%) and body weight (BW -7%) and a tendency for plasma Ca in LowCaP diet during the starter diet only when broilers received ORGACID (Water x CaP; $P=0.016$, 0.031 , 0.07 respectively). Daily water consumption (DWC) tended to be

decreased in LowCaP diet without effect on average daily feed intake (ADFI; $P=0.06$). Bone mineral content (BMC) was decreased in LowCaP diet only when broilers received INORGACID (Water x CaP; $P=0.047$). Similar results were obtained during the grower phase with ORGACID in which birds tend to be affected more from the LowCaP in terms of final BW, ADFI and FCR (Water x CaP; $P=0.06, 0.08, 0.07$ respectively). The BMC and plasma P were reduced in LowCaP diet during grower phase (-15% and -16%; CaP, $P<0.001$). During the finisher phase, ORGACID-NCaP consumed more than ORGACID-LCaP (Water x CaP; $P=0.05$). Also, LCaP have a lower impact (-10%) on BMC/BW with INORGACID than ORGACID (-18%) and no acidification (-28%; Water x CaP; $P=0.05$) and plasma P was reduced in LCaP (-12%; $P<0.001$). The evolution of BMC over time showed that birds receiving INORGACID did not have a reduced BMC when fed a LCaP diet at day 30 (Time x CaP x Water; $P<0.001$). Therefore, the P supply by INORGACID (phosphoric acid) was sufficient to cope the dietary Ca and P reduction effect on bone mineralisation. Nevertheless, the water acidifiers tested did not improved growth performance and we have even seen a reduction with ORGACID in LCaP. Further studies are needed to understand their effects and optimize water acidification practices.

Key Words: bone; broiler; calcium; phosphorus; acidifiers

414P Fenugreek (*Trigonella foenum-graceum* L.) seeds and *Bacillus* based direct-fed microbials alter the immune response and cecal microbiota in broiler chickens. Deependra Paneru², Guillermo Tellez-Isaias³, Emmanuel Asiamah¹, Walter Bottje³, Jayant Lohakare^{1*}, ¹University of Arkansas at Pine Bluff, Pine Bluff, Arkansas, United States, ²University of Georgia, Athens, Georgia, United States, ³University of Arkansas, Fayetteville, Arkansas, United States.

Two experiments were conducted to study the effects of fenugreek seeds (FS) and direct-fed microbials (DFM) on the growth performance, expression of ileal immune-related genes, and cecal microbiome in broilers. In the first experiment, day-old Ross male broilers ($n = 144$) were fed 0 g, 5 g, and 10 g FS per kg of diet during the starter (1 to 21 d) and finisher phase (22 to 42 d) (6 replicates/treatment with 8 birds each). Second experiment was conducted with FS and *Bacillus* DFM using day-old Ross male broilers ($n = 160$) arranged in 2×2 factorial with the inclusion of two levels of FS (0 g/kg and 5 g/kg) and two levels of DFM (0 g/kg and 0.1 g/kg) (5 replicates/treatment with 8 birds each). The increasing levels of FS linearly reduced ($P<0.05$) the growth performance of broilers during starter phase, but performance was unaffected during the finisher phase in experiment 1. For experiment 2, FS reduced ($P<0.05$) the BW, BW gain during the starter phase but did not affect it during the finisher phase. FS and DFM interaction effects were observed on BW in the finisher phase where BW was significantly higher in control than in other groups, but it

was similar to the 5 g/kg FS group added with 0.1 g/kg DFM. For the experiment 1, compared to the control, expression of interleukins (IL6 and IL8L2), β – defensins (AvBD9, AvBD10 and AvBD11), Caspase 6, Prostaglandin-endoperoxide synthase 2, and Interferon regulatory factor 7 were downregulated. For experiment 2, compared with controls, expression of interleukins (IL6 and IL8L2), avian β -defensins (AvBD9 and AVBD11), caspase 6, prostaglandin-endoperoxide synthase 2, and interferon regulatory factor 7 were downregulated with either FS or DFM, whereas AvBD10 was upregulated with DFM and downregulated with FS inclusion. In the first experiment, the alpha diversity analyses indicated that cecal microbiota diversity was significantly reduced in 5 g and 10 g FS groups compared to control group. Compared to the control group, the relative abundance of actinobacteriota and acidobacteriota was significantly decreased, whereas an increase in firmicutes was observed. In second experiment, alpha diversity analyses indicated that the cecal microbiota diversity was not different among the treatments and control group. Beta diversity analyses indicated that phylum bacteriodota was significantly reduced in 5 g FS with 0.1 g DFM group compared to the control ($P=0.012$). These findings indicate that FS inclusion in diet decreased growth performance, provoke an immunological response and influences the cecal microbiota. However, DFM did not influence growth performance but provokes the immunological response and alters the cecal microbial community in broilers.

Key Words: cecal microbiome; Broilers; immune response; *Bacillus*-DFM; Fenugreek

415P Carvacrol as alternative growth promoter: impact on animal performances, caecal metabolomic fingerprint and meat quality. Angel Alfonso-Avila^{1*}, Benedict yanibada¹, Jean-François GABARROU², Marie-Pierre Létourneau Montminy¹, ¹Laval University, Quebec, Quebec, Canada, ²Phodé, Tersac, France.

The inclusion of sensorial feed additives in broiler diets has been consolidated as nutritional alternative to antibiotic growth promoters (AGP). This study evaluated carvacrol (CAR) as an alternative to AGP and its impact on performance, caecal metabolites, and bone mineral content (BMC). A total of two-hundred-eighty-eight ten-days old were randomly placed in 24 cages ($n=12$ /cage) and assigned to 2 treatments: 1) CON (control), and 2) CAR (carvacrol) that were given during growing (10-21 d) and finishing (21-34 d) phases. Animals and feed were weighed at d10, 21, 28 and 34 to assess body weight (BW), average daily gain (ADG), feed intake (ADFI), and feed conversion (FCR). One broiler per cage was euthanized for sample collections. Meat quality test was performed in breast obtained at d34. Liquid chromatography coupled to mass spectrometry was used to perform untargeted metabolites profile of caeca content ($n=12$ /treatment). Bone mineralisation was investigated by scanning 2 carcasses of broilers on d34 with

a dual-photon X-ray absorptiometry equipment. Animal performance data was analysed using MIXED procedures of SAS and for metabolomic data analysis, Compound Discoverer 3.0 (Thermo, Waltham, USA) was used for processing and Simca (V13, Umetrics AB, Umea, Sweden) for statistical analysis. Results showed that during the last days (28 to 34d), greater BW (tendency, 2320 vs 2257 g; $P=0.06$), ADFI (196.2 vs 187.3 g/d; $P=0.004$) and ADG (117.7 vs 109.9 g; $P=0.01$) was observed in CAR compared to CON while FCR was not modified. Caecal metabolome analyse (variable importance in projections; VIP > 1.8) showed an increase of apocynin, pyrimidine, sedanoline, and a decrease of glycine-lysine dipeptide, arginine, taurocholic acid, and taurohyocholic acid in birds receiving CAR as compared with CON. These metabolites indicate implications of CAR on primary bile acids and amino acids (mainly dipeptides). The treatment CAR tent to improve (likelihood Chi-Square $P=0.09$) the locomotion (gait score) of chickens in comparison with CON. Meat redness (a^*) was reduced when animals received CAR (12.7 vs 13.7; $P=0.001$). Overall, these results showed that CAR supplementation induces an improvement in broiler performances and changes in caecal metabolism. In further steps we will study the plasma metabolome profile associated with CAR.

Key Words: broiler; carvacrol; growth promoter; metabolomic

416P Capsaicin as alternative growth promoter: impact on animal performances, caecal metabolomic fingerprint and meat quality. Angel Alfonso-Avila^{1*}, Benedict yanibada¹, Jean-François Gabarrou², Marie-Pierre Létourneau Montminy¹, ¹Laval University, Quebec, Quebec, Canada, ²Phodé, Tersac, France.

The use of antibiotics as growth promoter in broilers led to antibiotics resistance, hence the ban of this use. Based on their functional properties, sensory feed additives (SFA) have been introduced as a nutritional alternative to antibiotic growth promoters (AGP). This study evaluated SFA [capsaicin (CAP)] as growth promoter and its impact on performance, caecal metabolites, bone mineral content (BMC). A total of two-hundred-eighty-eight ten-days old ROSS 308 broilers were randomly placed in 24 cages ($n=12/\text{cage}$) and assigned to 2 treatments: 1) CON (control without SFA and 2) CAP (capsaicin) that were given during growing (10-21 d) and finishing (21-34 d) phases. Animals and feed were weighed at d10, 21, 28 and 34 to assess body weight (BW), average daily gain (ADG), feed intake (ADFI), and feed conversion (FCR). One broiler per cage was euthanized for sample collections. Meat quality test was performed in breast obtained at d34. Liquid chromatography coupled to mass spectrometry was used to perform untargeted metabolites profile of caeca content ($n=12/\text{treatment}$). Bone mineralisation was investigated by scanning 2 carcasses of broilers on d34 with a dual X-ray absorptiometry equipment. Animal performance data was

analysed using MIXED procedures of SAS and metabolomics data were analysed using Compound Discoverer 3.0 (Thermo, Waltham, USA) for data processing and Simca (V13, Umetrics AB, Umea, Sweden) for statistical multivariate analysis. At d34, greater BW (4.6%, $P<0.001$) and ADFI (3.8%, $P<0.001$) was observed when animals received CAP compared with CON. Similarly, animals fed CAP diet increased ADFI and ADG as compared with CON (4.5% and 7.2%, $P<0.01$) and decreased FCR (3.6%, $P<0.001$). Caecal metabolome analyses (variable importance in projections; VIP > 1.8) showed an increase of cytidine, glutamic-lysine, gamma-glutamylglutamic acid aspartylphenylalanine, and a decrease of valylvaline, taurocholic acid, taurochenodeoxycholic acid, lysine, arginine and serotonin in birds receiving CAP as compared with CON. These metabolites indicate implications of CAP on primary bile acids and amino acids. In comparison to CON, CAP increase BMC/kg of BW (8.5%; $P=0.01$) and tended to increase bone mineral density (tendency; 6.8%; $P=0.06$). Lower liver weight (6.6% BW; $P=0.03$) was also observed with CAR compared with CON. Regarding meat quality parameters, meat redness (a^*) was reduced when animals received CAP (7.5%; $P<0.001$). Overall, these results showed that capsaicin supplementation induces an improvement in broiler performances and changes in caecal metabolism. In further steps we will study the plasma metabolome profile associated with CAP.

Key Words: broiler; Capsaicin; growth promoter; metabolomic

417P Growth performance, carcass composition responses to supplementation of a gluconeogenic feed additive to corn-soybean meal diet for Ross 708 broiler chickens. Shravani Veluri^{1*}, Oluyinka Olukosi¹, ¹University of Georgia, Athens, Georgia, United States.

A 42-day floor pen study was conducted to determine the effect of supplementing a gluconeogenic feed additive (GA) in reduced-energy diets for broiler chickens. A total of 720 Ross 708 straight run broiler chicks at day old were allocated to three treatments with 12 replicates of 20 birds per pen. The birds in the pens were balanced as much as possible to have equal number of male and female chicks. Treatments include a positive control (PC) diet adequate in all the nutrients according to Ross 708 nutrient recommendation. A negative control (NC) diet in which energy is reduced by 50 kcal/kg relative to the PC diet. Reduced energy was obtained by removal of 10 g/kg soy oil in feed formulation. The third diet was NC diet supplemented with GA feed additive. The diets were fed in starter (0 to 14 d), grower (14 to 28 d), and finisher (28 to 42 d) phases and feed intake (FI), weight gain (BWG), and FCR calculated for all the phases. On d 42, jejunal tissue was collected from 2 birds per replicate for histomorphology; and carcass processing was done for 3 randomly selected birds from each pen. Data were analyzed

using the MIXED model procedure of SAS. There was no significant treatment effect on BWG, FI, and mortality in starter, grower, and finisher phases. There was no significant treatment effect on FCR for starter (d 0 to 10) and finisher (d 28 to 42) phases, however, FCR was improved ($P < 0.05$) in the NC+GA compared to other treatments in the grower (d 10 to 28) and starter plus grower (d 0 to 28) phases. There was no significant treatment effect on villus height, crypt depth, villus width, but villus height to crypt depth ratio tended ($P = 0.076$) to be greater for PC and NC+GA compared to NC treatment. There were no significant treatment effects for carcass yield and cuts measured which included fat weights and yield of breast, wings, and thighs. Conclusions from this study are that the NC+GA diet improved FCR in grower phases and has the potential to improve villus histomorphology and hence is beneficial in diet in which dietary energy was reduced by removal of some of the oil content.

Key Words: carcass yield; growth performance; glucogenic feed additive; jejunal histomorphology

418P Effect of conditioning temperature on pellet quality measured with Holmen and ASABE S269.5 methods. Jose Vargas^{1*}, Joseph Gulizia¹, Susan Bonilla¹, Jose Hernandez¹, Leopoldo Almeida², Sureerat Thuekeaw¹, Patricia Soster¹, Cesar Escobar Lobo¹, Orlando Fiallos¹, Marco Reina Antillon¹, Wilmer Pacheco³, Charles Starkey¹, ¹Auburn University, Auburn, Alabama, United States, ²UFPR, Curitiba, Paraná, Brazil, ³Auburn University, Auburn, Alabama, United States.

In the poultry industry, pelleting is a widely applied feed manufacture technology that has been associated with improvements in feed conversion and weight gain of birds, as it increases feed intake and reduces feed wastage and nutrient segregation. However, these benefits can only be achieved if pellets retain their physical structure during handling and transportation. Therefore, the ability of the pellets to withstand disintegration has become crucial and has been measured using Pellet Durability Index (PDI). A trial was conducted to evaluate the effect of three conditioning temperatures (80, 85 and 90°C) on the PDI of a commercial broiler diet. A corn-soybean meal-based diet with a fat content of 2.77% was blended for 150 seconds (30 seconds dry cycle and 120 seconds wet cycle) using a twin shaft mixer (Model 726, Scott Equipment Co., New Prague, MN) to produce the mash diet. Then, the diet was conditioned at 80, 85 and 90°C, maintaining a retention time of 40 seconds and pelleted through a 4.0-mm pellet die using a pellet mill (Model 1112-4, California Pellet Mill Co., Crawfordsville, IN) at a constant production rate of 636 kg/h. Pellet samples were collected and immediately cooled with ambient air. Pellet samples were analyzed using three procedures. Two procedures were the ASABE method S269.5 without and with the addition of five 1.3 cm hex nuts in the tumbler compartment to simulate handling and transportation. The third method was a Holmen NHP 100

(TekPro Ltd, Norfolk, GBR) set to a timer of 30 seconds. All data were analyzed as a one-way ANOVA using the GLM procedure of JMP PRO 15 and means were separated using Tukey's HSD with statistical significance considered at $P \leq 0.05$. Pellet durability improved ($P \leq 0.05$) with each 5°C increase of conditioning temperature when measured with all three procedures. Diets analyzed using the ASABE method S269.5 without and with the addition of hex nuts conditioned at 90°C had a significantly higher ($P \leq 0.05$) PDI (88 and 86%) compared to diets conditioned at 85°C (85 and 81%), which was significantly higher ($P \leq 0.05$) than the PDI of diets conditioned at 80°C (83 and 78%). Similar results were obtained for the Holmen NHP 100 as diets conditioned at 90°C had a significantly higher ($P \leq 0.05$) PDI (83%) compared to diets conditioned at 85°C (75%), which was significantly higher ($P \leq 0.05$) than the PDI of diets conditioned at 80°C (69%). The results of this study indicate an improvement of PDI with gradual increases of 5°C of conditioning temperature, maintaining retention time, mixer fat, and throughput constant.

Key Words: amino acid digestibility; apparent metabolizable energy; corn

419P Response of broiler chickens in the finisher and withdrawal phases to different levels of metabolizable energy and available lysine. Timothée Guillou^{1*}, Carole Margetyal¹, Olivier Gestin², Edith Crenn², Claire Launay¹, ¹ADM Animal Nutrition - Neovia, Chierry, France, ²ADM Animal Nutrition - Wisium, Saint-Nolff, France.

The present study was designed to test the interaction between metabolizable energy (ME) and available lysine (avLys) on broiler growth performance during a 14-d period and carcass traits. A total of 1,440 Ross 308 chicks were allotted to 40 floor pens (24 birds/pen). All birds received a common feeding program from 1 to 20 d of age. At 21 d, 6 dietary treatments in a 2 x 3 factorial design were assigned including 2 levels of ME, low and high (3,020 and 3,160 kcal/kg AMEn from 21 to 28 d; 3,080 and 3,220 kcal/kg AMEn from 29 to 35 d) and 3 contents of avLys, low, medium and high (0.93, 1.00 and 1.07% from 21 to 28 d; 0.90, 0.97 and 1.04% from 29 to 35 d). Over the 21 to 35 d period, all diets were formulated in order to get similar av(M+C):avLys and avThr:avLys ratios. Body weight (BW) and feed intake (FI) were recorded, weight gain, feed conversion ratio (FCR) calculated, as well as Lys and ME efficiency ratios. Thirty-five birds per treatment were selected at 35 d. Those birds received respective experimental diets until slaughter at 40 d. Carcass and cut part weights were measured. Data were analyzed using two-way ANOVA and means compared by Tukey test at 5% probability. No differences ($P > 0.05$) were observed in performance from 1 to 20 d, when common feeding program was given. During 21 to 35 d period, increasing ME level lowered ($P < 0.05$) FI from 115.2 to 114.0 g/d. A significant ($P < 0.05$) interaction between levels of ME and avLys was observed for weight gain and FCR. While birds

fed medium and high contents of avLys exhibited better FCR compared to those fed low content of avLys, the avLys-increase-associated benefits were more pronounced at the higher ME level. Also there was a nearly significant ($P = 0.08$) interaction for 35-d-old BW. A significant ($P < 0.01$) interaction between ME and avLys levels was observed for ME and Lys efficiency ratios. Increasing avLys progressively decreased the Lys efficiency ratio, the avLys-increase-associated detriments were alleviated at the higher ME level. Contrariwise, increasing avLys progressively increased the ME efficiency ratio, the avLys-increase-associated benefits were more pronounced at the lower ME level. Carcass and thighs weights were greater ($P < 0.01$) for broilers fed high ME level compared to those fed low ME level, there was no effect of ME on yields ($P > 0.05$). In addition, birds fed medium and high avLys had significant higher ($P < 0.01$) breast weights and breast yields than birds fed low avLys content. In conclusion, the feed conversion ratio can be easily managed by modulating energy and lysine contents in the diet. However, these factors must be manipulated strategically to improve profitability of broilers production.

Key Words: broiler chickens; energy; lysine; performance; carcass traits

420P Effect of conditioning temperature and production rate on moisture content and pellet durability index. Sureerat Thuekeaw^{1*}, Leah Smith², Susan Bonilla², Joseph Gulizia², Martha Rueda Lastres², Jeffery Escobar³, Jared Froetschner³, Wilmer Pacheco², ¹*Chulalongkorn University, Pathumwan District, Bangkok, Thailand*, ²*Auburn University, Auburn, Alabama, United States*, ³*Elanco Animal Health, Greenfield, Indiana, United States*.

Pellet quality influences performance and nutrient utilization of broilers and it is typically measured using either the ASABE method S269.5 or a Holmen tester. Pellet quality is important to improve feed efficiency and to reduce feed wastage and nutrient segregation. The objective of this study was to evaluate the effect of conditioning temperature and production rate on pellet quality measured by pellet durability index (PDI) and moisture content of the conditioned mash. The experiment consisted of a 3×2 factorial arrangement of 3 conditioning temperatures (60, 75, and 90°C) and two production rates of 636 and 409 kg/hr. A corn-soybean meal-based diet with 2.4% soybean oil addition was mixed in a twin shaft mixer, steam conditioned and pelleted with a 50-horsepower pellet mill. Pellet samples were collected throughout the run and immediately cooled. The following day PDI was analyzed using the ASABE method S269.5 with the addition of 5 hex-nuts to simulate pellet breakage during handling and transportation and with the Holmen NHP 100 (TekPro Ltd, Norfolk, UK) for 60 seconds. Data were statistically evaluated using the GLM procedure of JMP and means were separated by Tukey's HSD with statistical significance

considered at $P \leq 0.05$. Increasing conditioning temperature from 60 to 90°C increased ($P \leq 0.05$) moisture content of the conditioned mash from 15.34 to 17.16%. Decreasing production rate from 636 to 409 kg/hr increased ($P < 0.05$) PDI from 89.7 to 92.8% and from 51.2 to 63.6% when measured with the tumbler and Holmen NHP 100, respectively. Significant interactions ($P < 0.05$) were observed between production rate and conditioning temperatures on PDI; the greatest increase in PDI ($P < 0.05$) was observed when production rate was lowered from 636 to 409 kg/hr in diets conditioned at 60 and 75°C, but in diets conditioned at 90°C production rate did not influence PDI when measured with both methodologies. The results of this study demonstrated that increasing conditioning temperature and reducing production rate improves pellet durability of corn-SBM diets, which can have a positive effect on broiler growth performance.

Key Words: conditioning temperature; pellet quality; production rate; moisture

421P Effect of the inclusion of U.S. tannin-free grain sorghum varieties and canthaxanthin on growth performance of brown egg-type pullets. Alexa Johnson¹, Gracie Anderson¹, Santiago Sasia^{1*}, Mireille Arguelles-Ramos¹, Ahmed Ali¹, ¹*Clemson University, Clemson, South Carolina, United States*.

The objective of this study was to investigate the influence of partial replacement of corn with U.S. tannin-free sorghum, either with or without supplementation of canthaxanthin, on the growth performance of brown egg-type pullets. A total of 840 day-old Hy-line Brown chicks were housed in an environmentally controlled house. Treatment diets consisted of corn-based (CON), corn-high protein sorghum (HS), and corn- U.S. No. 2 sorghum (N2) diets, with sorghum replacing 50% of the corn and two levels of canthaxanthin (Carophyll[®], DSM Nutritional Products) inclusion (0 or 35 mg/kg). Birds were in a randomized complete block design in a 2×3 factorial arrangement with five pen replicates/treatment and 28 birds/pen. Pen weights and feed rejected were recorded at 1, 3, 5, 7, 9, 11, 13, 15, and 17 wks of age to calculate average daily feed intake (ADFI), body weight (BW), and average daily gain (ADG). Uniformity was calculated at 6, 8, 10, 12, and 14 days of age. Differences in measured parameters across weeks and between treatments were assessed using GLMM with Tukey's Post hoc test applied to significant results in R 3.3.1 (α set at 0.05). At 3 wk-of-age, birds fed the N2 diet had higher ADFI than those fed HS ($P = 0.05$). At one wk-of-age birds fed CON and HS were heavier than those fed N2 ($P < 0.05$). By 3 wk HS had a higher BW than N2 ($P < 0.05$). The ADG for CON and HS was higher at 1 and 3 wk-of-age ($P < 0.05$). No significant differences were observed in ADFI, BW, or ADG after 3 wk-of-age ($P > 0.05$) or flock uniformity for any of the ages evaluated ($P > 0.05$). In conclusion, the partial replacement of corn with tannin-free sorghum, either with or without

supplementation of canthaxanthin, did not affect the growth performance of brown egg-type pullets.

Key Words: growth performance; uniformity; sorghum; egg-type pullet; pigments

422P Influence of diet algae and corn distillers grain inclusion on broiler performance for a 28-42d finisher period. Savannah Wells Crafton^{1*}, Kenneth Nelson¹, Muhsin Anas², Mahinda Atapattu³, Craig Maynard¹, Michael Kidd¹, Garrett Mullenix¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Gadjah Mada University, Yogyakarta, Indonesia, ³University of Ruhuna, Matara, Sri Lanka.

In order to add to the body of literature pertaining to novel protein ingredient Microalgae, *Arthrospira platensis* (algae), an experiment was conducted to evaluate the diet algae and corn distillers grain (DDGS) inclusion on broiler performance for a 28-42d finisher period in Cobb CF05 male broilers. Previous research conducted at the University of Arkansas showed interactions between algae and DDGS. To investigate this interaction an experiment designed as a 2 x 2 factorial was used. Four dietary treatments were used in this study and consisted of diets containing algae at inclusion levels of either 0% and 2% and DDGS at inclusion levels of 0% and 8%. Diet inclusion of algae resulted in 3% reduction in soybean meal usage. All diets were iso-caloric and formulated to have similar amino acid profiles and CP level. Diets were fed to 384 male broilers, placed in eight replicate pens of twelve birds and live performance was assessed from d 28 to 42. At d 42, six birds from each pen were randomly selected and processed for evaluation of carcass traits and incidence of woody breast. All data were analyzed as a full factorial with a mixed model using JMP software with algae, DDGS, and algae x DDGS as fixed effects and block as a random effect. The F-protected Fisher's LSD test was used to separate means when $P \leq 0.05$. No significant responses were observed for algae, DDGS, and algae x DDGS influence on BWG, FI, and FCR or processing characteristics; ingredient source did not affect bird performance.

Key Words: Broiler; Protein; Algae; Novel

423P Effects of the energy concentration and the standardized ileal digestible lysine content of the diet on productive performance and egg quality of brown-egg laying hens from 18 to 41 weeks of age. R. Scappaticcio^{2, 1} L. Cámara¹, J. Herrera², Gonzalo Mateos^{1*}, A.F. de Juan¹, G. Fondevila¹, ¹Universidad Politécnica de Madrid, Madrid, Spain, ²Camar Agroalimentaria S.L., Toledo, Spain.

The influence of the energy concentration and the standardized ileal digestible lysine (DLys) content of the diet on egg production and egg quality was studied in brown-egg laying hens from 18 to 41 wk of age. The experimental design was completely randomized with 10 treatments organized as a 2 x 5 factorial with 2 energy

concentrations (2,750 and 2,800 kcal AMEn/kg) and 5 levels of DLys that varied from 0.66 to 0.78% and from 0.67 to 0.79% for the low and high energy diets, respectively. Each treatment was replicated 10 times and the experimental unit was an enriched cage with 10 hens for all measurements. To ensure that the DLys content of the diet was limiting hen production in all cases, all diets were formulated to exceed by at least 2 percent units the desired ratio between key indispensable AA and DLys content. Production data (feed intake, egg rate, egg weight, egg mass, FCR, and BW gain) were collected and analyzed by period (6 periods of 4 wk each), phase (pre-peak from 18 to 21 wk and peak from 22 to 41 wk of age), and cumulatively (18 to 41 wk of age). Egg quality traits (egg weight, Haugh Units, and shell resistance to breakage) were measured in 8 eggs randomly selected from each replicate for the last 2 d of each of the 6 experimental periods. The data were analyzed using the MIXED procedure of SAS with energy concentration and DLys contents of the diets as main effects. In addition, the effects of the DLys content of the diet on the variables studied were partitioned into its lineal (L) and quadratic (Q) components. From 18 to 21 wk of age (pre-peak phase), diet composition had limited effects on egg production. The number of days needed to reach 30% egg production, decreased linearly as the DLys content of the diet increased ($P = 0.096$). From 22 to 41 wk of age (peak phase) an increase of 50 kcal/kg diet increased egg weight ($P < 0.05$) and tended to increase energy intake ($P = 0.083$) and to improve FCR ($P = 0.074$). An increase in DLys from 0.665 to 0.785% improved linearly ($P < 0.001$) egg production, egg weight, egg mass, FCR, and ECR, and tended to increase BW gain ($P = 0.074$). Egg quality traits were not affected by diet, except shell strength that increased (L, $P < 0.05$) with increases in DLys. Cumulatively (18 to 41 wk of age), egg weight increased with increases in dietary energy and DLys ($P < 0.05$). In summary, an increase in energy and DLys content of the diet had limited effects on hen production during the pre-peak phase (18 to 21 wk of age) but improved most of the traits during the peak production phase (22 to 41 wk of age). In this phase, laying hens require at least 839 mg DLys/d to optimize egg production and egg size.

Key Words: egg quality; energy concentration; laying hen production; standardized ileal digestible lysine requirements

424P Assessment of the accuracy of NIRS technology to determine the proximal composition and amino acid content of commercial soybean meal samples. Laid Dardabou¹, Gonzalo Mateos^{2*}, L. Aguirre², M.A. Ibañez¹, ¹Polytechnic University of Madrid, Madrid, Spain, ²Universidad Politécnica de Madrid, Madrid, Spain.

In total, 30 commercial samples of soybean meal (SBM) from USA (n = 11), Brazil (n = 10), and Argentina (n = 9) were collected randomly from European feed compound plants by trained personnel. The DM, CP, amino acids, ether

extract after HCl hydrolysis (EEh), and CF were analyzed by wet chemistry (WCh) and NIRS. All wet analyses were performed in the same laboratory using official methods. NIRS values were determined by 4 specialized European laboratories (A, B, C, and D) using their own calibration models. Independently of the SBM origin, the average and range of WCh values (as fed bases) were 88.7 and 85.6 to 91.1% for DM, 46.2 and 44.2 to 49.8% for CP, 2.41 and 1.40 to 3.30% for EEh, 4.66 and 4.10 to 5.20% for CF, 2.91 and 2.75 to 3.06% for Lys, 0.63 and 0.53 to 0.68% for Met, and 0.67 and 0.59 to 0.75% for Cys. Bias, slope, and unexplained error were the statistics used to investigate the accuracy of WCh vs. NIRS technology, including comparisons among the 4 laboratories. The analysis was supported by a scatter plot of the ratio of NIRS to WCh (di) vs. NIRS values (ni) on a logarithmic scale, including bias confidence limits (BCL), limits of agreement, and any other trend that could be detected in the scatter plot. DM content was properly determined by the 4 laboratories, with 95% of the ratios within the 0.981 to 1.031. CP values were close to reference values, with a mean ratio between 0.992 and 1.048. The BCL variation among labs implied that the underestimation and the overestimation for the CP varied between 2% and 5%, respectively. For EEh and CF, the variability of the ratio ranged from 0.49 to 2.37 and from 0.47 to 1.29, respectively. The scatter plot trends showed that the magnitude of the error detected depended on the variable measured. For Lys, NIRS values were similar among companies, with laboratory "C" showing the lowest rate and an underestimation of around 7%, whereas for laboratory "D" the overestimation was as high as 7%. For Met, laboratory "C" had with a slightly higher prediction error than all the others, with overestimation and underestimation being of 6% and 4%, respectively. The error for Cys varied by 4% and 9%, for underestimation and overestimation, respectively. NIRS data from laboratories "A" and "D" were similar to WCh data with a SSEP of 0.02. In summary, the data showed that NIRS technology is a valid alternative to WCh to estimate the chemical constituents of commercial SBM. However, its accuracy according to the analyzed component, the company that developed the calibration data, and factors, such as the country of origin of the SBM, that affects the chemical composition of the samples.

Key Words: amino acids; soybean meal; near-infrared spectroscopy technology; wet chemistry ; proximal chemical analyzes

425P Not presented.

426P Standardized ileal amino acid digestibility of high-oleic full-fat soybean meal in broilers. Muhammad Ali¹*, Maria Alfaro-Wisaquillo¹, Gustavo Quintana-Ospina¹, Michael Joseph¹, Danny Patino¹, Ondulla Toomer², Thien Vu², Lina Peñuela-Sierra¹, Edgar Oviedo-Rondón¹, ¹North Carolina State University, Raleigh, North Carolina, United

States, ²USDA, SEA ARS, Raleigh, North Carolina, United States.

High oleic oil soybeans can produce high-quality protein meals, and their fatty acid content can improve several metabolic processes in broilers. However, its amino acid (AA) digestibility has not been determined. The objective of this study was to determine the apparent (AID), and standardized ileal AA digestibility (SID) of high oleic full-fat (HO-FF) soybean meal (SBM) compared to normal oleic full-fat (NO-FF), extruded expeller (NO-EE), and solvent extracted (SE) SBM in broilers. The extruded SBM were all processed under similar conditions, and particle size was uniformized by roller-milling to obtain a geometric mean of 915-950 μ m. The HO-FF contained 38.18% crude protein (CP), 18.21% fat, the NO-FF, 38.31% CP and 18.21% fat, the NO-EE 43.80% CP and 8.99% fat, and the SE SBM 47.15% CP and 2.57% fat. A nitrogen-free basal diet (NFD) was fed to one treatment with ten chicks/cage to determine basal endogenous losses (BEL). This NFD contained 78% corn starch, 6.5% dextrose, 5% soybean oil, 3% Solka flock, and 1% Arbocel as fiber sources, plus vitamins and minerals. Titanium dioxide was used as an inert marker to calculate AA digestibility. The test diets contained 57.5% of the basal NFD and 42.5% of each of the four SBM sources. In this experiment, 272 Ross 708 male broilers were placed in 40 battery cages and assigned to five treatments, with eight replicates per treatment, using a completely randomized design. A common starter diet was provided to all the chickens for 14 d. All chickens had similar ($P>0.05$) BW, feed intake, and FCR before offering the NFD and the four test diets. Experimental diets were provided in the mash during nine days of adaptation. Chickens were euthanized with CO₂ at 24 d, and the ileal distal portion contents were collected, frozen, and freeze-dried. The BEL were similar to values found within the literature. The AA AID of the HO-FF SBM was similar ($P<0.001$) to the NO-FF SBM but lower than the observed in NO-EE and SE SBM for Lys, Met, Val, Leu, Ile, Arg, Asp, Ala, Glu, Gly, and Ser. However, the AID of Thr, Trp, and Cys of HO-FF was similar to the NO-EE but always lower than SE SBM. The SID in the HO-FF SBM was 88.10, 87.60, 81.73, 82.02, 83.18, 82.05, 85.07, and 91.29% for Lys, Met, Thr, Val, Leu, Ile, Trp, and Arg, respectively. These SID for HO-FF were lower than SE and NO-EE SBM but slightly higher than NO-FF SBM. The AA and CP digestibility reduced ($P<0.001$) from SE, NO-EE, HO-FF to NO-FF SBM. Values of SID of all AA for SE and NO-EE SBM were similar to those reported in the literature for high-protein and high-quality SBM. In conclusion, the SID of AA from HO-FF is similar to other full-fat SBM and lower than normal oleic extruded expeller and solvent extracted SBM.

Key Words: broilers; digestibility; soybean meal; Amino acids; high oleic soybean meal

427P Effects of corn particle size on growth performance, gastrointestinal development, carcass indices and intestinal microbiota of broilers. Lei Yan^{1*2} Sha An^{1,2} Zunzhou Lv^{1,2} Jianshu Zhuo¹, Yong Li^{1,2} Mingan Choct³, Zhengguo Wang^{1,2} yuming guo⁵, Guilian Zhou^{1,2}, ¹Shandong New Hope Liuhe Group Co., Ltd., Qingdao, China, ²New Hope Liuhe Co., Ltd., Beijing, China, ³University of New England, Armidale, Australia, ⁴Key Laboratory of Quality Control for Feed and Products of Livestock and Poultry, Ministry of Agriculture, Chengdu, China, ⁵China Agricultural University, Beijing, China.

This experiment was conducted to investigate the effects of different corn particle sizes on growth performance, gastrointestinal development, carcass processing yields and intestinal microbiota of caged broilers. One-day-old Ross 308 broilers were randomly divided into 8 treatments with 10 replicates per treatment and 30 broilers per replicate. The particle size for the finely ground (FG) corn was 485 µm. For the coarsely ground (CG) corn it was 1693 µm for d1 to 13, 2310 µm for d14 to 23, and 2409 µm for d 24 to 37. The experiment lasted 37 days. Feed and water were provided ad libitum. The results showed as follows: birds fed diets with the FG corn between d 1 to 13 and CG corn between d14 to 37 had increased body weight, daily gain and feed intake ($P < 0.05$). Birds fed diets with CG corn between d 24 to 37 had heavier relative weight of gizzard at d 38 ($P < 0.05$). Birds fed the FG corn diets between d 1 to 13 and the CG corn between d 14 to 37 had an increased carcass yield and a thigh relative weight at d 38 ($P < 0.05$). The intestinal microbiota was significantly affected by different corn particle sizes. The relative abundance of *Lactobacillaceae* in the intestinal tract of broilers was significantly decreased ($P < 0.05$), *Peptostreptococcaceae* was increased ($P < 0.05$) in birds fed with the CG corn between d1 to 37. The relative abundance of *Acinetobacter* was significantly increased in birds fed the FG corn between d1 to 37 ($P < 0.05$). In conclusion, the use of FG corn in the starter phase and CG corn in the grower and finisher phases was beneficial to growth performance, gastrointestinal development and intestinal microbial structure of broilers reared in cages.

Key Words: broilers; growth performance; intestinal microflora; corn particle size; carcass indices

428P Field traits, nutrient composition and digestible amino acids of different types of corn for poultry. Cristina Simíques¹, Catarina Stefanello^{1*}, Juliano Vidal¹, Janine Sarturi¹, Isadora Laber¹, Daniel Soares¹, Carlos Mallmann¹, ¹Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil.

This study aimed to evaluate the effects of the endosperm texture on nutritional composition and on field traits of different types of corn intended for poultry. A total of 212 corn samples were sourced from experimental field plots located in the western region of the Paraná state, Brazil, and separated into 4 groups according to the endosperm texture

classification: dent (n=31), flint (n=51), semi-dent (n=60) and semi-flint (n=70). Crop yield, thousand grains weight (TGW) and damaged grains were measured. Samples (500 g) were milled with a 0.5 mm sieve in an ultra-centrifugal mill and then homogenized. Nutritional analyses were carried out by reading the spectra of the samples by near infrared spectroscopy. The spectra were originated using the calibration curves from the AMINONir[®] advanced program. The following nutritional values were predicted: dry matter (DM, %), crude protein (CP, %), ether extract (EE, %), ash (%), starch (%), total P (mg/kg), phytic P (mg/kg), total and digestible amino acids (AA, %) for poultry, gross energy (GE, kcal/kg) and metabolizable energy (ME, kcal/kg) for poultry. These values were corrected for an 87% DM basis. Data were submitted to analysis of variance using the software Statgraphics Centurion XVI. Significance was accepted at 5% and different means of types of corn were separated by Tukey Test. Dent corn presented higher percentage of damaged grains ($P < 0.01$) than other types whereas flint corn had the lowest crop yield ($P < 0.001$). There were no effects ($P > 0.05$) of corn types on TGW. Dent corn presented higher ($P < 0.01$) starch concentration than flint corn. Flint corn presented higher EE than dent corn ($P < 0.05$) and the highest CP ($P < 0.001$) and total Met, Cys, Thr, Arg, Ile, Leu and Val. The digestibility of AA for poultry was different among types of corn, where digestible Cys, Thr, Arg, Ile, Leu, Val, His, Phe, Gly, Ser, Pro, Ala, and Glu were higher ($P < 0.0001$) in flint corn compared to dent, semi-dent and semi-flint types. Also, digestible Met and Lys were higher in dent and flint corns ($P < 0.0001$), compared to semi-dent and semi-flint corns. Dent and semi-dent corns presented lower gross energy than flint corn ($P < 0.001$). Metabolizable energy for poultry was higher in flint corn than dent ($P < 0.05$). There were no effects ($P > 0.05$) of corn types on concentrations of total and phytic P. In conclusion, there are differences in field traits and nutritional composition of corn depending on the characteristic of its endosperm, which are factors that should be considered by the corn and poultry nutrition chains.

Key Words: digestibility; starch; poultry nutrition; NIRS; endosperm texture

429P The effect of high and low energy and crumble quality on growth performance in broilers. Nelsa Beckman^{1*}, Khairy Jenkins², Allison Blomme¹, Haley Ottot¹, Kara Dunmire¹, Charles Stark¹, Chad Paulk¹, ¹Kansas State University, Manhattan, Kansas, United States, ²North Carolina A&T, Greensboro, North Carolina, United States.

Broilers are often fed crumbles in early production to improve growth performance. Thus the objective of this study was to determine the effect of metabolizable energy (ME) concentration in diets and if removal of crumble fines influences the response on growth performance of broilers. At hatch, a total of 300 one day old male broilers (Cobb 500,

initial BW 42.1g) were used in an 18-day study. Broilers were housed in 3 Petersime batteries with *ad libitum* access to feed and water. Treatments were randomly assigned to 60 cages within location block, resulting in 10 cages per treatment with 5 broilers per cage. Treatments were arranged in a 2×3 factorial of ME content (3,034 and 2,969 ME, kcal/kg) and crumble fines in a randomized design. Diets were pelleted and crumbled. Crumbles were fed without sifting (NS) or sifted using either a screen with 1.5 mm openings (removed particles <1,532 µm) or a screen with 0.86 mm openings (removed particles <864 µm). Data were analyzed as a randomized complete block design using the GLIMMIX procedure of SAS 9.4 (Cary, NC). There was no evidence of an interaction between crumble fine removal and ME or main effect of ME. Body weight gain (BWG) and total feed intake (TFI) increased ($P<0.05$) when broilers were fed crumbles sifted with a 1.5 mm screen (776 g and 910 g, respectively) compared to NS (719 g and 867 g, respectively) and crumbles sifted with a 0.86 mm screen (742 g and 889 g, respectively). Feed conversion ratio (FCR) improved ($P<0.001$) when broilers were fed crumbles sifted with a 1.5 mm screen (1.17) compared to those fed crumbles not sifted (1.21) and sifted with a 0.86 mm screen (1.20). In conclusion, broilers fed crumbles sifted with a 1.5 mm screen showed an improved FCR and an increased BWG and TFI regardless of the energy content of the diet. Increasing ME from 2,969 to 3,034 kcal/kg did not influence growth performance of broilers.

Key Words: broiler; crumble; energy; growth performance

430P Effect of conditioning temperatures and retention times on crumble quality. Cesar Escobar Lobo^{1*}, Jose Vargas¹, Orlando Fiallos¹, Marco Reina Antillon¹, Joseph Gulizia¹, Susan Bonilla¹, Jose Hernandez¹, Leopoldo Almeida¹, Sureerat Thuekeaw¹, Patricia Soster¹, Wilmer Pacheco¹, Charles Starkey¹, Kenneth Macklin¹, ¹Auburn University, Auburn, Alabama, United States.

Crumbles are obtained by using corrugated rollers to break whole pellets into smaller articles that are easier to consume by small chicks. Two experiments were conducted to evaluate the effect of conditioning temperature (CT) and retention time (RT) during conditioning on crumble quality. For both trials, a corn-soybean meal-based diet with 2.77% fat content was mixed for 150 seconds (30 seconds dry cycle and 120 seconds wet cycle) using a twin shaft mixer to obtain a basal meal diet. For trial 1, the basal diet was conditioned at 80, 85 and 90°C, with 40 seconds RT. For trial 2, the basal diet was conditioned at 80°C, with a RT of 40, 80 and 120 seconds. Both diets were pelleted through a 4.0-mm pellet die. After pellet cooling, diets were processed through a twin roll crumbler with manual adjustment (Model 624SS, California Pellet Mill Co., Crawfordsville, IN). A composite sample per treatment was formed by collecting and mixing multiple samples of crumble collected at evenly spaced intervals during packaging. The composite sample from each treatment was used for

crumble quality analysis. From each composite sample, 10 replicates of 100 grams were analyzed using either the manual shaker sieve to separate particle depending on their size (<1,000 µm, 1,000 to 2,000 µm, 2,000 to 3,000 µm, and >3,000 µm) or the automatic *DuraTap*TM sieve shaker using US sieves number 6 (3,360 µm), 8 (2,380 µm), 16 (1,190 µm), and 30 (590 µm). Samples were shaken for 1 minute regardless of method used. Data was analyzed using one-way ANOVA (GLM) (significant $P \leq 0.05$), means were separated using Tukey's HSD in SPSS® software. When crumble quality was measured with the manual sieve shaker, diets conditioned at 90°C had greater ($P \leq 0.05$) percentage of fine particles (<1,000 µm) (23 vs. 18 and 15%) compared to diets conditioned to 85 and 80°C. Furthermore, increasing RT to 120 seconds increased ($P \leq 0.05$) the percentage of fine particles compared to RT of 80 and 40 seconds (23 vs. 17 and 17%). When crumble quality was analyzed with the *DuraTap*TM, increasing RT to 120 seconds increased ($P \leq 0.05$) the percentage of particles smaller than 1,190 µm compared to diets with RT of 80 seconds (32 vs. 25%) with 40 seconds RT being intermediate (28%). The lowest ($P \leq 0.05$) percentage of fine particles was obtained when diets were conditioned at 80°C (24 vs. 32 and 31%). In conclusion, increasing CT and RT had a negative effect on crumble quality as measured by the percentage of particles smaller than 1,000 µm.

Key Words: conditioning temperature; crumble; quality; retention time

431P Growth performance of broilers fed soluble and insoluble fiber in interaction with the probiotic *Bacillus amyloliquefaciens* CECT 5940. Catarina Stefanello^{1*}, Carine Adams¹, Jessica Agilar¹, Valeria Biselo¹, Otoniel Souza¹, Vinicius Teixeira², Nadia Yacoubi³, ¹Federal University of Santa Maria, Santa Maria, RS, Brazil, ²Evonik Brazil Ltda, Nutrition & Care, São Paulo, Brazil, ³Evonik Operations GmbH, Hanau-Wolfgang, Germany.

An experiment was conducted to evaluate the effects of soluble and insoluble fibers in diets for broilers until day 35 of age. The effect of fiber in interaction with a probiotic on growth performance of broilers chickens was also investigated. A total of 672 one-day-old male Cobb 500 slow feathering broilers were used. Dietary treatments were distributed in a 2 x 2 factorial arrangement, with 6 replicates and 28 broilers each, using a completely randomized design. A three-phase feeding program was used where corn-soy basal diets with analyzed ingredients and practical levels of energy and amino acids were formulated with rye or defatted rice bran as insoluble or soluble fiber sources, respectively (inclusions were 10, 15, and 20% in starter, grower, and finisher diets, respectively). Diets were not supplemented or supplemented with the probiotic *Bacillus amyloliquefaciens* CECT 5940 (1,000 g/ton). Growth performance was evaluated per week and from day 1 to 14, 1 to 21, 21 to 35, and 1 to 35. Data were submitted to one-way analysis of variance using the MIXED procedure of

SAS. Means were compared by the Tukey test when effects of dietary treatments were significant at 5%. No interaction was observed between fiber and probiotic. The BW gain and FCR improved when broilers were fed diets with higher insoluble fiber as well as when diets were supplemented with the probiotic ($P < 0.05$). From day 1 to 35, the BW gain increased by 4% in broilers fed high insoluble fiber compared to the high soluble fiber diet ($P < 0.05$). When diets were supplemented with probiotic from day 1 to 35, BW gain and FCR were improved by 3.4 and 5.2%, respectively compared to the non-supplemented diet ($P < 0.05$). In conclusion, these results demonstrated that the probiotic *Bacillus amyloliquefaciens* CECT 5940 resulted in improved growth performance of broilers. Broilers fed diets formulated with inclusions of an insoluble fiber ingredient had improved performance.

Key Words: broiler; feed additive; performance; probiotic; alternative ingredient

432P Use of rice waste products in ostrich diets. Pouyan Malekinezhad^{1,2}, Laura Ellestad^{2*}, Nazar Afzali², Seyed Homayoun Farhangfar², Ali Nakhaei², ¹University of Georgia, Athens, Georgia, United States, ²University of Birjand, Birjand, Iran (the Islamic Republic of).

Proper use of agricultural and human food waste in animal diets and identification of alternative, inexpensive food sources for livestock should be critical priorities in developing strategies to reduce costs and improve the environmental sustainability of production operations. The rice industry generates large quantities of waste, including straw, husk, ash, bran, and broken rice. To investigate the effect of using rice waste products (RWP) in ostrich diets on performance, economic indicators, carcass traits, and blood parameters, and experiment with five treatments (0, 10, 20, 30, and 40% RWP) and 50 ostriches (*Camelus Struthio*) was performed. The birds were housed individually in outdoor paddocks of 30 m² and had free access to feed and fresh drinking water. Each treatment group was composed of 10 birds (n=10 birds/diet), and an individual bird was considered a replicate. The chemical composition of RWP was 93.93% DM, 12.03% Ash, 11% CP, 5.90% Fat, 5.20% Fiber, and 3130 (Kcal/kg) ME. The treatments were control (0% RWP) and four levels of RWP (10, 20, 30, or 40%) in the diet. At 8 and 9 months of age, feed intake was measured, and all birds were weighed after fasting for 12h to determine average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). At the end of the experiment (9th month), blood samples were obtained from the brachial vein of all ostriches for analysis of serum cholesterol, total protein (TP), albumin, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride (n = 10 birds from each group). After this, all birds from each treatment were euthanized and subjected to analysis for carcass yield, and meat cut yield. Before analysis, data normality was investigated by the Shapiro-Wilk test. Then the mixed

procedure was used for performance data and the general linear model for blood biochemical and carcass traits (SAS). With 20% and 30% RWP, FI and BWG increased ($p \leq 0.05$), and FCR was not affected ($p > 0.05$). Carcass yields and relative weight were not significantly decreased with RWP substitution ($P \leq 0.05$). With an increasing percentage of RWP, HDL and TP increased, and cholesterol and LDL decreased significantly ($p \leq 0.05$); other parameters were not influenced. The average production cost and efficiency index were not significantly different between treatments ($P > 0.05$). These data demonstrate that up to 30% substitution with RWP can be used without significant negative growth effects; therefore, RWP can be introduced as a suitable ingredient for ostrich diets.

Key Words: by-product; ostrich; rice; waste products

433P Effect of Pistachio waste as an alternative source of diet and its impact on performance, and some blood parameters of ostrich. Pouyan Malekinezhad^{1,2}, Laura Ellestad^{1*}, Nazar Afzali², Seyed Homayoun Farhangfar², Ali Nakhaei², ¹University of Georgia, Athens, Georgia, United States, ²University of Birjand, Birjand, Iran (the Islamic Republic of).

On average, 70 to 80 percent of the total cost of raising poultry is related to feed. Therefore, any factor that affects the composition of the diet in a way that reduces cost and either improves or does not negatively impact growth performance will benefit production efficiency. The use of agricultural and food industry waste in animal feed can reduce the cost of feed and lessen the amount of food for human consumption used in poultry diets. Pistachio (*Pistacia vera* L.) hull is the most significant by-product of the pistachio industry that is accumulated every year at harvest time and can lead to environmental pollution. In this study, we investigate the effect of adding pistachio waste (PW) containing rigid and green hulls in the diet of young ostriches. A total of 40 six-month-old ostrich chicks (*Camelus Struthio*) with an initial weight of 50.5±1 kg were used to study the inclusion of PW on growth performance and blood biochemical traits of ostriches from 7 to 9 months of age. The birds were divided into four groups, housed individually in outdoor paddocks of 30 m², and had free access to feed and fresh drinking water. Each treatment group was composed of 10 birds, in which each bird was considered a replicate. The chemical composition of PW was 93.62% DM, 6.12% Ash, 12.28% CP, 7.03% Fat, 18.12% Fiber, and 2690 (Kcal/kg) ME. The treatments were control (0% PW) and three levels of PW in the diet (10, 20, or 30%). At 8 and 9 months of age, feed intake was measured, and all birds were weighed after fasting for 12h to determine average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). At the end of the experiment (9th month), blood samples were obtained from the brachial vein of all ostriches for analysis of serum cholesterol, total protein (TP), albumin (ALB), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and

triglyceride (TG) (n = 10 birds from each group). Data were analyzed using the mixed model procedure of SAS. Results showed that 10% addition PW did not differ significantly in feed consumption from the control treatment. In comparison, the levels of 20 and 30% of PW added to the diet caused a significant increase in feed consumption compared to the control treatment. Adding 20% of PW to the diet increased the ADG of ostriches compared to the control treatment ($p \leq 0.05$). FCR was not significantly different among treatments ($p > 0.05$). Adding 20 and 30% PW reduced serum cholesterol and LDL compared to control ($p \leq 0.05$), and TP, ALB, TG, and HDL did not significantly differ among treatments ($p > 0.05$). In conclusion, adding PW did not hurt ostrich growth performance and could be used as a substitute in the diet at up to 30% to reduce the cost of feed.

Key Words: average daily gain; blood biochemistry; Ostrich; Pistachio

434P Maternal fish oil alters adipose development through transcriptional regulation in the broiler chick embryo. Minjeong Kim^{1*}, Usuk Jung¹, Jeanna Wilson², Jenny Drnevich³, Brynn Voy¹, ¹*The University of Tennessee, Knoxville, Tennessee, United States*, ²*University of Georgia, Athens, Georgia, United States*, ³*University of Illinois, Urbana, Illinois, United States*.

Enriching the hen diet in long chain omega-3 polyunsaturated fatty acids (n-3 PUFA), especially eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) can reduce body fat deposition in chickens. We previously demonstrated that enriching the broiler-breeder hen diet in fish oil, a rich source of EPA and DHA, reduced adiposity and adipocyte size in chicks after hatch compared to a corn oil-based hen diet, suggesting that maternal fish oil diet induces sustained molecular effects in adipose tissue development in the broiler chicks. Here we tested the hypothesis that maternal fish oil (MFO) specifically alters adipose development through transcriptional regulation in the broiler chick embryo. Cobb 500 broiler-breeder hens (n=30/diet) were fed diets containing 3% fat from fish oil or soybean oil (SO, n-6 PUFA control) for four weeks. Fertilized eggs were randomly collected and incubated to embryonic ages E14, E16, and E20 (n=20/diet/age). At each age, subcutaneous fat pads were dissected and fixed in 4% paraformaldehyde for histological analysis. Adipose sections stained with H&E were imaged with an EVOS XL Core Imaging System and analyzed adipocyte size using ImageJ (V 1.52a; NIH.gov). Diet effects were evaluated using Student's t-test ($p < 0.05$). At E16, adipose tissues were snap-frozen and used to isolate RNA. Genome-wide RNAseq was used to identify transcriptional effects of MFO on adipocyte development. Trimmed reads were mapped to the *G. gallus* transcriptome derived from genome bGalGal1.mat.broiler.GRCg7b (NCBI, Annotation 105) using Salmon (v1.4.0). After normalization, RUV (Peixoto

2015) was used to remove effects of correlated data structures, and limma-trend was used for differential expression based on FDR. Histological analysis revealed that MFO significantly reduced adipocyte size, increasing the frequency of small adipocytes at each age examined ($p < 0.05$). Expression levels of 323 genes were significantly influenced by MFO, with 210 upregulated and 113 down-regulated genes compared to MSO (FDR $p < 0.05$). Gene set enrichment analysis revealed that MFO significantly affected expression of sets of transcription factors known to control tissue development as well as genes involved in fatty acid binding, synthesis, and metabolism. In addition, MFO increased expression of four members of the Iroquois homeobox genes that recently have shown to regulate adipogenesis in humans and rodents. These data indicate that EPA and DHA influence expression of the genome in developing broiler chick embryos. They also reveal mechanistic pathways that can be manipulated through the hen diet to limit fat accretion and influence body composition in broilers, and potentially other species.

Key Words: Embryo; Broiler; Fish Oil; Maternal Diet; Adipose Development

435P Assessment of energy and amino acid diet formulation in growing Ross broilers. Matheus Costa^{1*}, Kenneth Nelson¹, Savannah Wells Crafton¹, Craig Maynard¹, Garrett Mullenix¹, Mahinda Atapattu¹, Michael Kidd¹, ¹*University of Arkansas, Fayetteville*.

This study investigated the effect of low and high protein diets formulated based on net energy (NE) and metabolized energy (ME) on live performance and carcass characteristics of 7-32 day old Ross 708 broilers. The NE equation can be described as the ME minus heat increment. Once NE is predicted, important factors must be taken in consideration in formulation, since formulation based on NE can enhance the energy efficiency and provide closer energy needs for production and maintenance. Birds were placed in four tunnel ventilated, solid-sided colony houses. Lighting and temperature regimes followed the primary breeders recommendation, and feed and water were provided *ad libitum*. Three hundred and eighty-four females and male broilers (Ross 708) were randomly allocated in four poultry houses with two houses for females and two houses for males (one hundred and ninety-two birds per house). Each house had sixteen pens (3 x 4 feet) of birds which were fed one of four different experimental treatments (n=4 or 8). The treatments diets were 1) (22.5% CP, 3,100 kcal/kg ME, 2,553 kcal/kg NE). 2) (20.2% CP, 3,100 kcal/kg ME, 2,553 kcal/kg NE). 3) (22.5% CP, 3,049 kcal/kg ME, 2,500 kcal/kg NE). 4) (20.2% CP, 3,049 kcal/kg ME, 2,500 kcal/kg NE). House x treatment interactions occurred for male live performance; thus, male live performance was not combined by house. Differences in male and female live performance as affected by diet did not occur. Although processing differences were minimal, carcass fat in females was affected by diet. Carcass fat was

decreased in female broilers fed the highest CP diet ($P < 0.03$), and an interaction between CP and energy occurred for carcass fat ($P < 0.10$) in female broilers. The interaction revealed similar carcass fat of energy formulation in female broilers fed the high CP diet, but when CP was reduced, NE formulation reduced the carcass fat.

Key Words: Broilers; Low protein; Net energy; High protein; Metabolized energy

436P Evaluation of the effects of dietary *Uronema* spp. on the growth performance, feed intake and feed conversion ratio of broiler chickens. Hannah Shinnerl^{1*}, Katherine Ho¹, Tryg Lundquist¹, Ruth Spierling², Ike Kang¹, Darin Bennett¹, Mark Edwards¹, ¹California Polytechnic State University, San Luis Obispo, San Luis Obispo, California, United States, ²MicroBio Engineering Inc, San Luis Obispo, California, United States.

The poultry industry supplies meat and eggs to the increasing global consumption of high-quality protein. Feed represents 60 to 75% of production cost. Increasing costs and lower availability of corn and soybean meal (SBM) accentuate the need for alternative feedstuffs. Microalgae produced as a byproduct of CO₂ capture of coal-fired power generation have emerged as an alternative source of dietary energy, essential amino acids, carotenoids, and omega-3 fatty acids. Our objective was to evaluate the inclusion of microalgae on broiler chicken production performance. 360 one-day-old Ross 708 broilers with an average initial body mass of 43.87g were sorted and randomly allocated to 3 dietary treatments with 8 replicate pens (1.2m²) of 15 birds/pen. Dietary treatments were isonitrogenous, isoenergetic, consisted of a corn-SBM diet and graded levels (0, 2.5%, 5%) of microalgae (*Uronema* sp.) and were available *ad libitum*. Body mass and feed intake were measured weekly over 6 weeks. Data were analyzed with a repeated measures ANOVA. Diets were the main effect, weeks were the repeat, and pen was the experimental number. Body mass did not differ ($P > 0.5$) until weeks 5 ($P = 0.0006$) and 6 ($P = 0.0018$) when birds consuming the 5% algae diet weighed more than the control, resulting in an overall difference in mass ($P = 0.026$). Body mass gain was greatest in the 5% treatment and lowest in the control ($P = 0.0018$). Feed intake did not differ among treatments ($P > 0.05$). Microalgae treatments had a lower feed conversion ratio (FCR) than the control during weeks 4 ($P = 0.039$) and 6 ($P = 0.0017$) although overall, no difference in FCR was detected ($P > 0.05$). These results are consistent with previous findings of increased growth of broilers fed microalgae compared to a negative control. Additional research is required to evaluate microalgae as a premium feedstuff, and its effects on broiler health and carcass quality.

Key Words: broiler; microalgae; feed intake; growth; performance

437P Carryover effects of breeder flock age (pullet or late) and feeding strategy (choice or no choice) as it influences 14-61d bird beak capacity, performance and processing. Maria Alvarenga^{1*}, Pratima Adhikari¹, Joe Moritz², Kelley Wamsley¹, ¹Mississippi State University, Starkville, Mississippi, United States, ²West Virginia University, Morgantown, West Virginia, United States.

This study is a continuation from a previous study that utilized a 2 Breeder Flock Age (BFA; Pullet, 27±1 wk, PBFA; and Late, 51±1 wk, LBFA) x 2 Feeding Strategy (FS; choice (CH) or no choice (NC)) factorial arrangement of treatments within a randomized complete block design from 0-14 d. The objective of the current study was to evaluate d 14-61 carryover effects of BFA and starter FS on performance, bird beak capacity (BBC) and processing. A total of 800 Ross x Ross 708 males were used (20 birds/BFA/pen; n=40 pens), of which, 8 bird/pen were tagged for BBC. It was observed that LBFA had increased BW and BBC vs. PBFA at d 0-14; however, BFA did not impact feed particle size (FPS) consumed (FPSC), regardless of FS. Also, BBC did not impact FPSC. The FS implemented from 0-14 d included CH (10 different FPS from 38-4750 µm in separate feeders within a pen, made from a common diet) or NC (common crumble, ~1050-1200 µm, within 10 separate feeders in a pen). For chicks fed CH, average FPSC ranged from 1600-2400 µm; when given NC, average FPSC ranged from 1700-2600 µm. Common pelleted diets for each subsequent growth phase were provided to all birds from 14-62 d. On d 62, eight birds/pen were randomly selected for processing. Beak measurements continued on d 32, 46 and 61 using the same birds measured from 0-14 d and a digital caliper. Performance metrics were observed on d 32, 46 and 61. Similar to d 0-14 results, BW for PBFA was lower than LBFA on d 32 ($P = 0.0023$); however, no difference was found at d 46 ($P = 0.6711$). A BFA x FS interaction occurred for d 61 BW ($P = 0.0182$), where LBFA with 0-14 d CH had a higher BW than PBFA fed CH and LBFA fed NC; all were similar to PBFA fed NC. For d 14-32 and 46 d FI, LBFA had increased FI compared to PBFA ($P < 0.05$); no difference was observed from 14-61 d. Day 0-14 FS did not impact any metric observed in carryover phases (i.e., FI, BW, FCR; $P > 0.05$). However, BFA x FS impacted live BW of birds processed ($P = 0.0039$); LBFA with 0-14 d CH and PBFA with NC had the highest BW compared to LBFA fed NC; however, all treatments were similar to PBFA fed CH from 0-14 d. Measured processing metrics were not impacted by BFA or 0-14 d FS ($P > 0.05$). In general, only BFA impacted BBC ($P < 0.05$), with PBFA < LBFA; BBC measurements impacted decreased as birds aged. As such, significance was found for length (d 32, 46), opening (d 32), and height (d 46); no difference at d 61 ($P > 0.05$). These data demonstrate impact of BFA on 61 d performance and BBC; as birds age, differences lessen. Also, depending upon FS from 0-14 d, bird FPS preference ranged from 1600-2600 µm, which overall had no negative carryover impact on performance.

Key Words: Performance; Broiler; Processing; Beak capacity; Feed particle size

438P Potential role of curcumin as an adaptogen: implications on animal performances. Angel Alfonso-Avila^{2*1}, Jean-François Gabarrou³, Marie-Pierre Létourneau-Montminy¹, ¹Université Laval, Quebec, Quebec, Canada, ²CRSAD, Québec, Quebec, Canada, ³Phodé, Tersac, France.

Adaptogens are plant-based products that help cells to mediate or adapt physiological stress. Curcumin (CUR) possesses antioxidant and anti-inflammatory properties, a functional characteristics that has stimulated the addition of this sensory feed additive (SFA) as an alternative to antibiotic growth promoters (AGP). This study was intended to evaluate CUR as an alternative to AGP on animal performance and cecal metabolites. However, an unpredicted stress condition resulting from a high temperature (during the first 3 to 5 days of age, based on observational body temperature data) has made it possible to document the potential adaptogenic role of CUR in broilers. Three hundred day-old (Ross 308 male) broiler chicks were allocated into two pens (n=150), and received one of the two treatments 1) control (CON; without SFA) or 2) CUR (Force 6 Poultry, 60 ppm, Phodé Laboratories, France) during the starter phase (0-10 d). Then, ten-days old broilers (n=288) were randomly placed in 24 cages (n=12/cage) and assigned according to their respective treatments and were fed during growing (10-21 d) and finishing (21-34 d) phases. Animals and feed were weighed at d10, 21, 28 and 34 to assess BW, average daily gain (ADG), feed intake (ADFI), and feed conversion (FCR). One broiler per cage was euthanized for sample collections. Animal performance (grower and finisher phases) data was analysed using MIXED procedures of SAS. On the basis of limited data without statistical analysis, the average of body weight (BW) at d10 was 134.6 and 177.4 g, respectively CON and CUR. These performances represent only 42 and 55% of performances objectives for Ross 308, numerical differences that are probably explained in part by the impact of heat stress. Results showed an improved ADG during growing and finishing phases when animals received CUR vs CON (13% and 6% respectively; $P=0.001$). Thus, BW at 20 and 34 d were higher with CUR (633 vs 520 g; $P=0.045$ and 1988 vs 1838 g; $P=0.001$). Birds fed CUR have higher ADFI during the three phases ($P<0.05$). The FCR was increased during 20-28d when animals received CUR (9.4%; $P<0.001$). In sum, these results showed that CUR supplementation allow an improvement in broiler performances in a condition of stress. In further steps we will study the plasma metabolome profile associated with CUR as well as inflammation and oxidative stress based on sample taken during the trial.

Key Words: Broiler; curcumin; Adaptogens

439P Comparison of dietary inorganic and organic selenium responses on growth performance, gut health, and selenium accumulation in coccidiosis-infected broiler chickens. Samiru Wickramasuriya^{1*}, Inkyung Park¹, Hyun Lillehoj¹, ¹USDA-Agricultural Research Service, Beltsville, Maryland, United States.

The present study was conducted to investigate and compare the effect of dietary inorganic and organic selenium (Se) supplementation on growth performance, gut health makers, and breast selenium concentration in coccidiosis-infected broiler chickens. A total of 168 one-day-old Ross broilers were randomly allocated into one of four treatments in a 2×2 factorial arrangement with respective factors being, two different *Eimeria* challenges and the two different selenium diets (SS: Inorganic Se as sodium selenite and SY: organic Se as Selenized yeast; 0.3 mg/kg). Chickens in respective *Eimeria* challenge treatment groups were infected with *E. acervulina*, *E. tenella*, and *E. maxima* oocysts mixture (15,000 oocysts/chicken) on day 16. Six replicate cages (7 chickens each) were allocated per each treatment. Growth performance was measured on days 16, 22, and 24. On day 22, mucosa from duodenum, jejunum and caeca were collected to analyze the tight junction gene expressions. Breast muscle samples were collected to analyze the Se concentrations on day 24. There were no interactions ($p > 0.05$) between dietary selenium sources and coccidiosis on growth performance, tight junction gene expression, and tissue selenium accumulation of broiler chickens from hatch to 24 days of age. Greater ADG and body weight ($p > 0.05$) were observed with the chicken fed SY compared to SS diets from 1-16 days. Regardless of Se treatment, *Eimeria* infection reduced ($p > 0.05$) the weight gain and feed intake which commensurate with lower feed efficiency in broiler chickens. Dietary SY favorably improved ($p < 0.05$) tight junction genes expression in duodenum and jejunum compared to SS-fed chickens. However, SS-treated group showed better ($P < 0.05$) tight junction gene expression in caeca compared to SY chickens. Among Se sources, broiler chickens fed SY had higher ($p < 0.05$) Se accumulation in breast muscle compared to SS treatment. These results confirmed the higher efficiency of organic Se compared to inorganic Se for muscle Se enrichment, growth and gut health improvement of broiler chickens regardless of coccidiosis.

Key Words: broiler; coccidiosis; gut health; selenium; selenium accumulation

440P Evaluating the impact of organic chromium on hepatic phospholipid fatty acid molecular species, transcription of genes associated with lipid metabolism and oxidative status in broiler chickens fed flaxseed. Ahmad Fraz^{1*2}, Massimo Bionaz¹, Gita Cherian¹, ¹Oregon State University, Corvallis, Oregon, United States, ²University of Florida, Gainesville, Florida, United States.

Flaxseed is a rich source of α -linolenic acid (18:3 n-3) and is used to enrich chicken tissues with n-3 fatty acids (FA). However, antinutritional factors in flaxseed compromise the live performance of birds coupled with increased oxidative stress. Chromium (Cr) is a trace element with antioxidant properties. It is hypothesized that Cr supplementation will affect the production performance, hepatic lipid profile, and transcription of genes associated with lipid metabolism and antioxidative stress response in broiler chickens fed flaxseed. Ninety (n=90), day-old Cornish cross chicks were fed a corn-soybean meal-based diet containing 0% flaxseed (Control), 10% flaxseed (Diet 1) and Diet 1 + 0.05% organic Cr (Diet 2) for 42 days. The chicks were kept in 18 pens with 5 chicks per pen. For all response variables, the effect of dietary treatments were compared separately using SAS 9.4. P-values were considered significant at ≤ 0.05 . The overall weight gain was higher in Diet 2 and Control than Diet 1 ($P < 0.05$). Total lipids, saturated FA, long-chain ($\geq 20C$, LC) n-6 FA were reduced while total n-3 FA and LC n-3 FA were higher in Diet 1 and Diet 2 liver than Control ($P < 0.05$). Hepatic phosphatidylcholine (PC) and phosphatidylethanolamine (PE) n-3 species (36:5, 38:6) were higher in Diet 1 and Diet 2 compared to Control ($P < 0.05$). On the contrary, n-6 species in PC (36:4, 38:4) and PE (38:4) were lower in Diet 1 and Diet 2 compared to Control ($P < 0.05$). Supplementing Cr led to reduction in PE n-6 species (36:4) compared to Control and Diet 1 ($P < 0.05$). Transcription of *FADS2* was higher in Diet 1 than Control and Diet 2 ($P < 0.05$). Transcription of other genes involved in *denovo* lipid synthesis (*FASN*, *ELOVL6*, *PPARA*, and *HMGCS1*) was not affected by the diets ($P > 0.05$). We detected a trend for higher transcription of genes involved in FA oxidation (*CPT1A* and *ACAA2*) in Diet 1 and Diet 2 than Control ($P = 0.08, 0.07$). An increase in the transcription of *GPK2* gene involved in antioxidative response was observed in Diet 2 than in Control ($P < 0.05$). Lipid peroxidation products measured as thiobarbituric acid reactive substances were reduced in Diet 2 than Control ($P < 0.05$) and were similar to Diet 1 ($P > 0.05$). In conclusion, flaxseed supplementation enhances total and LC n-3 FA while reducing total lipids, saturated and n-6 FA in the liver. Supplementing Cr in flaxseed-based diets enhanced live performance, reduced n-6 FA species in the hepatic PE, and demonstrated a tendency to enhance the transcription of genes involved in FA oxidation and antioxidant stress response.

Key Words: liver; flaxseed; chromium; genes; n-3 fatty acid molecular species

441P Bioavailability assessment of manganese sources compared to manganese sulfate. Agathe Romeo¹, Romain Bouvet², Céline Bernard², Alessandra Monteiro^{1*}, Christophe Alleno², ¹Animine, Meythet, France, ²Zootests, Ploufragan, France.

For all animal species, including broilers, manganese (Mn) is an essential trace element. It is involved in bone formation

and mitigates locomotor problems. The objective of the study was to compare some manganese oxide (MnO) sources, from different manufacturing processes, with the standard manganese sulfate (MnSO₄). A total of 360 one-day-old female chickens (Ross 308) were randomly allocated to one of 9 dietary treatments (8 replicate pens/treatment, 5 chicks per pen) according to initial BW and continuously controlled over 35 d. Dietary treatments were negative control (NC) diet without Mn supplementation (35 ppm of native Mn) and 8 additional diets in which 30, or 80 ppm of Mn from 4 different sources were added to the NC diets. Sources tested were MnSO₄, standard MnO, purified MnO (ManGrin®, Animine) and MnO₂. At the end of the experiment, 12 broilers per treatment were sacrificed. Tibia and liver were taken in 8 animals per treatment (1 per pen) for Mn concentration analysis. Data were subjected to variance analysis (ANOVA). Linear regression was used to assess the bioavailability by using Mn in liver as a function of Mn intake. Statistical significance and tendencies were considered at $P \leq 0.05$ and $0.05 < P \leq 0.10$, respectively. Increasing dietary Mn doses increased Mn concentration significantly in bone: from 6.1 mg/kg for NC, to 6.8 and 8.1 mg/kg for 30 and 80 ppm of supplemented Mn, respectively ($P < 0.01$). Values of Mn in bone for MnO₂ (5.95 mg/kg) were similar to values obtained with the negative control. For Mn concentration in liver, the purified MnO showed a tendency ($P = 0.057$) for higher values (2.82 g/kg) than the NC (2.23 g/kg) and to other oxide sources: 2.49 g/kg for standard MnO and 2.41 g/kg for MnO₂. Compared to MnSO₄, values were statistically the same (2.61 g/kg). The relative bioavailability of Mn sources was estimated using the MnSO₄ as the reference source: 157% for the purified MnO, 66% for the standard MnO, and 33% for the MnO₂. In conclusion, the manganese oxide sources showed different bioavailability values. These differences could be related to the physicochemical characteristics of the products, as it was demonstrated with zinc oxide sources.

Key Words: Broiler; Manganese; Bioavailability

442P Effects of different dietary supplementation of source and level of zinc on intestinal morphology and its related gene expression in liver and jejunum of broilers. Mohammad Pilevar^{1*}, Oluyinka Olukosi¹, ¹University of Georgia, Athens, Georgia, United States.

A total of 378 one-day old of Cobb 500 were used to investigate the effect of different sources and levels of Zn on gene expression of antioxidant enzymes in liver, amino acids and Zn transporters in jejunum and ileal histomorphology of broiler chickens. The chicks were assigned to 9 treatments in a randomized complete block designed for 21 days with 7 replicates and 6 birds per cages. Diets were in a 4×2 factorial arrangement plus one basal diet (0 mg supplemental Zn/kg). The factors were 4 sources (ZnSO₄·7H₂O, hydroxychloride Zn, Zn-glycinate, and Zn-lysine-glutamate) and 2 levels of Zn (25 mg/kg and 100 mg/kg). At the end of feeding trial, liver mRNA levels of

glutathione peroxidases (GPXs), superoxide dismutases (SODs); and also, mRNA levels of amino acids and Zn transporters in jejunum were determined by quantitative real-time PCR. In addition, villi height and width and crypt depth were measured in the ileum. There were no significant effects comparing the basal diet to all other 8 dietary treatments. Zn source \times level interaction was observed for villus width with diet receiving 25ppm Zn as Zn-glycinate having wider ($P < 0.05$) villi at day 21. Similarly, Zn source \times level interaction ($P = 0.044$) was observed for jejunal expression of ZIP8 gene, with birds receiving 25 ppm Zn-glycinate diet having the lowest expression compared to other diets. Birds receiving inorganic Zn sources tended ($P = 0.051$) to have higher jejunum ZnT10 mRNA, compared to organic sources. At day 21, chicks fed the diet supplemented with low level of Zn-glycinate had higher GPX3 mRNA level in jejunum compared to other treatments ($P < 0.05$). The gene expression of amino acids transporters in jejunum was not influenced either by level or source of Zn. In conclusion, the low level of organic Zn negatively affected a Zn transporter gene expression but increased mRNA level of an antioxidant enzyme in liver. Therefore, it can be concluded that the organic and inorganic Zn sources influenced the bird's performance via different means.

Key Words: broilers; gene expression; Zinc; organic; inorganic

443P Impact of water electrolytic balance in broilers.

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Geographical location has an influence on water mineral profile. This study investigated the impact of water mineral profile on water and feed consumption, growth performance, bone mineralization and moisture content of excreta. Six hundred and twenty-four chicks were housed at 13 birds per cage in 48 cages for 34 d. The birds were selected according to their initial weight and assigned per block to 1 of the 4 treatments: 1) Acid (ACI; -1,3 mEq/L); 2) Alkaline (ALK; +5,1 mEq/L); 3) Neutral (NEU; +0,1 mEq/L); 4) Acid⁺⁺ (ACI⁺⁺; -3,3 mEq/L). The treatments are based on the electrolyte balance (EB) of water, which was determined according to water mineral concentration and the following equation: $\text{Na} + \text{K} - \text{Cl}$ (mEq/L). Broilers were fed a unique three-phase feeding program whose EB was 235, 225 and 183 mEq/kg of DM for the starting (1-10 d), growing (10-21 d) and finishing period (21-34 d), respectively. Water and feed offered were measured every day and refusals at the end of each phase. Bone mineralisation was determined by scanning 5, 3 and 2

carcasses of broilers on d10, d21 and d34, respectively with a dual-photon X-ray absorptiometry equipment. Tibia samples were also taken (1 broiler per cage) on d34 to measure minerals contents. Finally, moisture in excreta of 6 cages per treatment was evaluated 5 times during trial (days 7, 14, 16, 22, 34). Animal performances were analyzed using mixed model through JMP Pro 13 (SAS, 2021), considering treatment as fixed effects, and block and cage as random effects. Bone parameters were analyzed using MIXED model procedure of SAS (SAS 9.4). Tukey test was used for multiple comparison between treatments. During growing phase, treatment ACI reduced feed intake as compared with NEU and ACI⁺⁺ (89.75 vs. 92.87, 92.69 g per day; $P=0.02$). Average daily gain was also lower with ACI in comparison with ALK and ACI⁺⁺ (70.65 vs. 73.32, 73.23 g per day; $P=0.02$). As compared with ACI, NEU broilers increased water consumption (160.73 vs. 166.97 mL per day; $P= 0.04$). This last effect tended to continue during the finishing period ($P=0.09$) and feed conversion, during the growing period, and weight at d21 tended to be less for ACI broilers ($P=0.06$ and $P= 0.07$, respectively). However, no difference was detected between treatments for the final weight. No impact of treatments was observed for the moisture in excreta at any time. Finally, ACI broilers had a 6.7% higher bone mineral content of body on d34 than NEU broilers (0.01237 vs. 0.01154 g/kg of BW; $P=0.02$) without any treatments effect on the tibia mineral content. According to the EB of drinking water in broilers, this could be an element to be considered in the calculation of the dietary EB.

Key Words: broiler; excreta moisture; water electrolyte balance; bone mineralisation

444P Performance of Ross 708 Broilers Fed Organic or Inorganic Zinc Sources in the Presence of an Antagonist (Inositol Hexaphosphate) Zack Lowman^{1*}, Kari Estes¹, Jeffre Firman², ¹Balchem Corporation, New Hampton, New York, United States, ¹Balchem Corporation, New Hampton, New York, United States, ²Missouri Contract Poultry Research, Boonville, Missouri, United States.

Trace minerals are a crucial component of poultry diets for birds to achieve maximal performance. Chelated forms of minerals, also known as organic trace minerals (OTM), are often used in animal diets as a partial replacement for conventional inorganic trace mineral (ITM) sources due to protection from antagonists and thus increased bioavailability. The objective of this experiment was to evaluate the performance of broilers supplemented with OTM and ITM in the presence of an antagonist (inositol hexaphosphate; IP6). For this trial, 0.3% IP6 was added to the diets to replicate a natural challenge. Male Ross 708 birds ($n = 20$ birds/pen; 12 pens per treatment) were fed 1 of 6 corn-soybean meal-based diets that contained the equivalent of 40 ppm supplemental zinc (Zn) from either an OTM or ITM source: 1) Zn sulfate (positive control), 2) Zn sulfate+IP6 (negative control), 3) KeyShure Plus+IP6, 4)

KeyShure+IP6, 5) Zn amino acid complex+IP6, or 6) Zn hydrochloride+IP6. Body weight was measured at hatch and at 3 and 6 weeks of age. Feed intake and mortality were recorded throughout the 49-day experiment. Feed conversion was calculated at 3 and 6 weeks of age. Feather and paw scores were recorded at 21 and 49 days. Breast yield was measured on day 49. Data were analyzed as a one-way ANOVA using the general linear model in Minitab. Means were separated using Fisher's LSD test. No differences were observed in any of the parameters at 3 weeks of age. At 6 weeks of age there was a significant depression in BW gain with the addition of IP6 to the Zn sulfate diet (2.96kg vs 3.16kg). No differences were observed in feather or paw scores across treatments. At the conclusion of the trial, there were no differences in mortality, gain or intake across the treatments. However, mortality adjusted feed:gain was significantly higher ($P \leq 0.05$) for the Zn amino acid complex and lowest for the KeyShure and Zn hydrochloride treatments with the remaining treatments being intermediary. Breast yield was not statistically different between the Zn amino acid complex treatment (21.5%), the two control treatments (20.7%) and the KeyShure Plus treatment (20.8%). In this experiment, few differences were observed in broiler performance between the different Zn sources in the face of the antagonist model used.

Key Words: antagonist; zinc; organic trace minerals; trace minerals; chelated minerals

445P 2022 Survey of U.S. and Canadian broiler vitamin supplementation rates. Nelson Ward^{1*}, Doug Teitge¹, ¹DSM Nutritional Products, Ringoes, New Jersey, United States.

Vitamins in ingredients vary in level and digestibility, and are less reliable sources to meet intended dietary targets. Therefore, poultry feeds are supplemented with manufactured vitamin sources. This U.S. and Canadian (CA) survey was completed to determine vitamin supplementation rates in broiler feeds, and to assess variation across individual fortification rates among nutritionists. Over 96% of the U.S. broiler production (nearly 9.5 billion broilers) was accounted for in this survey. CA representation included six major broiler feed companies. Data were calculated on a pure vitamin basis to exclude carriers and other components. Summaries were tabulated as four broiler growout phases based on bird age: starter (S; d1-18), grower (G; d19-28), finisher (F; d29-42), withdrawal (WD; >d42). By phase, summaries included an overall mean, as well as averages for the highest and lowest 25% fortification rates for each of 12 vitamins. Coefficients of variation (CV) was an estimate of the consistency of supplementation rates. Comparisons were made with the 1994 National Research Council (NRC) and the DSM Vitamin Supplementation Guidelines. Average U.S. vitamin premix addition rates were 0.53, 0.44, 0.36, and 0.33 kg/MT, respectively, for S, G, F, and WD. For CA, this was higher at 1.53 (S), 0.96 (G), 0.82 (F), and 0.80 (WD)

kg/MT. U.S. and CA mean vitamin supplementation rates exceeded NRC recommendations. Pyridoxine was marginally the exception for G and F diets. Vitamin D in commercial feeds was higher than NRC by a factor of 14 to 18-fold. In all feeds, vitamins A, D, E, and K (menadione) represented the greatest disparity between commercial levels and NRC, with commercial being 3 to 6-fold higher. Vitamin levels declined from S to F by an average of 22%. When considering low 25% versus high 25%, the divergence increased from S to G to F: 43, 47, and 59%, resp. The high 25% was within the DSM Guidelines for S and G, excepting vitamins A and E, with less agreement in the F and WD phases. Vitamin E, folic, and biotin in S, and vitamin E and folic in G and F for CA exceeded U.S. levels by 20% of or more. No vitamin in CA premix was lower than in U.S. Variation (%CV) across supplementation rates was lowest for vitamin A and riboflavin, and highest for vitamin E and B12. Folic, biotin, pyridoxine, vitamin K and vitamin D were moderately variable by comparison. To summarize, vitamin fortification rates for broiler feeds in the U.S. and CA differed substantially from NRC, especially for fat soluble vitamins. Vitamin supplementation declined from S to WD. The difference between the low 25% versus high 25% rates was considerable. DSM Guidelines were in good agreement with the high 25% rates.

Key Words: Vitamins; broiler feed; NRC; DSM Guidelines; fortification

446P An evaluation of combination of 25-hydroxyvitamin D₃ and higher level of calcium and phosphorus in the diets on bone development and bone mineralization in layers. Dima White^{1*}, Chongxiao Chen², Woo Kim¹, ¹University of Georgia, Athens, Georgia, United States, ²North Carolina State University, Raleigh, North Carolina, United States.

Previously, our lab found 25-hydroxyvitamin D₃ (25OHD) with higher calcium & phosphorous (Ca/P) (25D+Ca) in the pullet diet increased the bone size and created more pores in the cortical bone. The higher-level addition of Ca/P filled up the pore space (open cavities within the cortical bone) created by 25OHD in the diet. We hypothesized that the additional Ca/P with 25OHD could improve and maintain bone structure and quality during the layer period. A study was conducted to explore the effect of 25OHD with higher Ca/P in the diets on bone structural development in layers. A total of 240 18wk Hyline W36 hens were randomly assigned to five treatment groups (5 trts x 8 reps x 6 birds/rep) and raised until 60wks. Dietary treatments were; 1) control vitamin D₃ (2,760 IU/kg), 2) control vitamin D₃ (2,760 IU/kg) + (31.25mg) HyD/ton (1/2 dose), 3) control vitamin D₃ (2,760 IU/kg) + (62.5mg) HyD/ton (full dose), 4) control vitamin D₃ (2,760 IU/kg) + (62.5mg) HyD/ton + high Ca/P, and 5) control vitamin D₃ (2,760 IU/kg) + high Ca/P. Ca was increased by 20% and P by 10 % for trts with increased Ca/P. At 60wk, femur bones were

collected and scanned using Micro-computed tomography for bone 3D structure analysis. The data were subjected to a one-way ANOVA using the GLM procedure, with means deemed significant at $P < 0.05$. The results indicated that 25D+Ca treatment had a trending of higher tissue volume in all bone parameters (total, cortical, trabecular and medullary bones) including higher ($P = 0.049$) bone mineral content (BMC) in all bone parameters compared to the other treatment. The 25D+Ca also had lower ($P = 0.042$) cortical volume of open pores, but a trending of higher closed porosity % compared to other treatments. Lastly the 25D+Ca had a trending of higher trabecular bone parameters (number, connectivity and connectivity density) compared to other treatments. The findings revealed that addition of 25D+Ca potentially improves volume in the volume of interest in tissue parameters of the bone. The lower cortical volume of open pores indicates that potentially 25OHD did grow a larger bone with the increased tissue volume and BMC, but at the same time higher Ca/P with 25OHD closed up the pore space with higher closed porosity. In conclusion, combination of 25D with higher Ca/P could maintain cortical bone quality in layers. The combination of higher bioactive form of vitamin D₃ and higher level of Ca/P could be a potential, (continuation from the pullet period), feeding strategy to improve & maintain layer bone quality.

Key Words: calcium; phosphorus; layers; vitamin D₃; 25-hydroxyvitamin D₃

447P Identification of biomarkers associated with *Campylobacter* colonization in broiler chickens.

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Campylobacter jejuni and *C. coli* are foodborne pathogens that cause human campylobacteriosis and affect around 95 million people worldwide. Majority of human campylobacteriosis is caused by *C. jejuni*, which is commonly present in the intestinal tract of chickens. Despite decades of research, human campylobacteriosis continues, and more than 80% of these infections are associated with consumption of poultry meat. Critically, the biological factors of the chicken gut that enable colonization of *Campylobacter* remain unknown. Identifying the host (i.e., chicken gut) factors involved in the colonization would enable us to develop intervention tools to control *Campylobacter* in the preharvest stage and aid in the prevention of *Campylobacter*-related foodborne illnesses and associated economic losses. We conducted a study in broiler chickens to identify the biomarkers critical for successful colonization of *C. jejuni* in broiler chickens. For this study, day-of-hatch broiler chickens were assigned to treatment groups (n=5 birds/group); (1) Negative Control – No challenge, (2) Positive Control - Challenged with

Campylobacter, and (3) EG treatment – *Campylobacter*-challenged chickens and supplemented with 0.125% eugenol (EG) in the water. We used eugenol, a phytochemical that showed reduction in *Campylobacter* colonization in chickens in our earlier studies. Birds were challenged with *Campylobacter* on day-7 and plasma samples were collected from each group on day 14 for conducting multi-omics analysis. Using a novel LCMS-based technology, we profiled biologically relevant molecules (protein, lipids, metabolites, and exosomes) that mediate *C. jejuni* colonization in the intestinal tract of broilers. Based on dimensionality reduction – PCA and Differential expression analysis - Biochemical-wise associations, Volcano plots analysis a total of 1216 analytes (275 compounds, 7 inorganics, 407 lipids, 527 proteins) were identified. PCA and UMAP were used to determine the global trends of the analytes. Based on PCA hierarchical clustering and UMAP analysis, we found that tauroursodeoxycholic acid, 5-hydroxyindole-3-acetic acid, guanine deaminase, an uncharacterized protein, taurine, were significantly deregulated in the EG group compared to the positive and negative control. In conclusion, we found several possible target molecules to control *Campylobacter* colonization and that could decrease its contamination in chicken meat. Further validation analysis is in process.

Key Words: broiler chickens; colonization; *Campylobacter*; phytochemicals; biomarkers

448P The effect of egg washing methods on shell bacteria levels and cuticle deposition for free-range flocks. Julie Bosland^{1*}, Becky Sartini¹, ¹University of Rhode Island, Newport, Rhode Island, United States.

Northeast small-scale and backyard poultry owners ($\leq 3,000$ birds) utilize a variety of different egg washing methods before sale or consumption. As indicated in the survey distributed in 2019 (n=166), the most frequently used washing methods were bucket washing (BW), antibacterial wipe (ABW), dish soap (DS), drybrusing (DB), water rinsing (WR) and water scrubbing (WS). Based on these survey responses, we hypothesized that different egg washing methods would decrease the total bacteria levels along with the cuticle deposition (CD) of the eggshell. Forty-five hens (White Leghorns and Plymouth Barred Rocks) were used in this study and housed at Peckham Farm (URI; Kingston, RI) in a mobile outdoor coop that allowed pasture access. Eggs were sampled for shell bacteria with Hygiena Microsnap™ Total testers and EnSURE luminometer (Hygenia, LLC, Camarillo, CA) and results were given in relative light units (RLU). The CD is the overall cuticle coverage quality on the eggs and was evaluated after each washing method by staining the eggs and taking measurements with a Minolta Colorimeter (Konica Minolta Sensing Americas, Inc.). Data was analyzed using SAS proc mixed procedure ($P \leq 0.05$). The following washing methods, BW (n=14), ABW (n=30), DS (n=33), DB (n=32) and WS (n=32) ($P \leq 0.01$) reduced shell

bacteria levels when compared to unwashed eggs. There was no difference observed in bacteria levels between WR (n=30) and unwashed eggs (n=188) ($P=0.98$). When comparing cuticle coverage, there was a difference between unwashed eggs (n=96) and eggs washed via BW (n=16), DS (n=27), DB (n=14) and WS (n=50) ($P\leq 0.01$). No difference was found in cuticle coverage between eggs washed with ABW (n=42) and WR (n=32) ($P\leq 0.42$) when compared to unwashed eggs. Based on the results, all washing methods except for WR showed a decrease in shell bacteria levels when compared to unwashed eggs. The integrity of the egg cuticle varied depending on the washing method. Washing methods that used soap/detergent or physical scrubbing decreased the cuticle on the egg whereas WR, and ABW had no effect on overall cuticle deposition. Based on these results, producers can use BW, ABW, DB and WS to wash eggs to reduce shell bacteria levels. The washing methods ABW and WR also maintained the integrity of the cuticle.

Key Words: small-scale; cuticle; Food safety; Free Range; egg washing

449P Effect of storage and time on egg quality and cuticle degradation. Julie Bosland^{1*}, Becky Sartini¹, ¹University of Rhode Island, Newport, Rhode Island, United States.

The egg cuticle is a protective (glycoprotein and antimicrobial) coat that covers the egg during oviposition and can protect the egg from *E. Coli* and *Salmonella typhimurium* penetration. We hypothesized that the time period (weeks) along with storage type would affect the cuticle deposition (CD), grade and weights of the egg. Brown and white eggs (N=72) were collected over a four-day period and sorted at random into twelve egg cartons, six eggs per carton. The twelve cartons are split into two groups based on the type of storage, either fridge (37-40 degree F) or room temperature (70-78 degree F) (RT). These egg cartons were then labeled weeks 1-6 for each storage group. Measurements were taken on the size, weight, and grade for each egg and documented as "Week 0 pre-measurements". Each week following, the same measurements were taken for each egg including the cuticle deposition (CD) on the eggshells. Data was analyzed using SAS proc mixed procedure ($P\leq 0.05$). There was a difference in CD and weights when comparing fridge and RT stored eggs ($P\leq 0.012$). Fridge stored eggs had better cuticle coverage and decreased weight difference when compared to RT eggs. There was no difference observed in the CD of eggs between weeks 1-6 ($P=0.8$), suggesting that there was no degradation in the cuticle quality over the course of 6 weeks in both fridge and RT stored eggs. White eggs had a greater CD than brown eggs ($P\leq 0.0001$) when compared between fridge and RT storage. There was no difference in the egg grade over weeks 1-6 in fridge and RT storage ($P=0.2$). Overall, eggs stored in the fridge for an extended time degraded at a slower rate than those stored at RT. Either storage method did not affect the CD and the cuticle

did not degrade over the course of the six-week storage period. Further replicates should be conducted to determine correlation between the type/length of storage and the cuticle and the overall egg degradation.

Key Words: egg grade; storage method; Egg Quality; cuticle; Storage Time

450P Incorporation of probiotics in wash water helps control *Salmonella* Enteritidis on shell eggs. Ragini Reddyvari^{1*}, Si Lu¹, Praveen Raja Kosuri¹, Mary Anne Amalaradjou¹, ¹University of Connecticut, Willimantic, Connecticut, United States.

Salmonella Enteritidis (SE) is a prevalent foodborne pathogen that is commonly transmitted through contaminated eggs. Once the egg is laid, SE can contaminate the eggs from various environmental sources including contaminated litter and fecal droppings. Therefore, decontamination of eggs is critical to food safety. Hence, we evaluated the efficacy of incorporating probiotics in wash water as an on-farm strategy to promote egg safety. We hypothesized that incorporation of probiotics in wash water will help reduce SE population on eggs. Further, since they occupy the same ecological niche as SE, probiotics can serve as a protective buffer against subsequent contamination and provide sustained antimicrobial effect. Freshly laid eggs (n=180) inoculated with a five-strain mix of SE (~8 log CFU/egg) were subjected to the different wash treatments including sterile water containing [no treatments (control), *Lactobacillus rhamnosus* NRRL-B-442 (LR; 8 log CFU/ml), *L. paracasei* DUP 13076 (LP) or *Hafnia alvei* (HA)] or chlorine (200 ppm). Eggs wash was performed either as a dip (32 or 42°C for 3 min) or spray application using a conventional air spray gun. Three eggs were sampled for *Salmonella* and probiotic populations after wash and over 14 days of refrigerated storage. The experiments were set out as a completely randomized design and three independent trials were performed. Data were analyzed using GraphPad (Version 9.3.1) and $p < 0.05$ was considered significant. Immediately after spraying, ~6 and 5.4 log CFU/ml of SE was recovered from the control and treated eggs, respectively. Beyond this initial reduction, probiotic sprays exerted a sustained antimicrobial effect against SE populations on eggs during refrigerated storage. For instance, SE was reduced by > 3 log CFU/ml and to below detection limits by day 7 and 14 of storage, respectively, while ~4 log CFU/ml was still recovered from the control and chlorine treated samples. With dipping, at the end of the 3 min wash period at 42°C, ~4 log CFU/ml of SE was recovered from the control and chlorine treated samples, with no further reduction observed during storage. On the other hand, washing with LR and LP reduced SE populations to below detection limits by day 7. Further, significant numbers of the probiotic were recovered from the eggs throughout storage. Overall, probiotic application by spraying or dipping significantly reduced SE populations

on eggs ($P < 0.05$). Therefore, incorporation of probiotics in wash water could potentially serve as a safe and organic-friendly antimicrobial strategy to promote egg safety.

Key Words: Salmonella Enteritidis; probiotics; egg washing; dipping; spraying

451P Aerotolerance of *Campylobacter jejuni* isolated from commercial broiler processing plants. Diksha Pokhrel^{1*}, Hudson Thames¹, Hailey Fugate¹, Li Zhang¹, Thu Dinh¹, Wes Schilling¹, Shecoya White¹, Anuraj Sukumaran¹, ¹Mississippi State University, Starkville, Mississippi, United States.

Broiler meat contaminated with *Campylobacter jejuni* is a leading cause of foodborne human gastroenteric infections in the United States. Although *C. jejuni* is considered a microaerophilic pathogen, which lacks the cellular mechanisms to survive in an oxygen-rich environment, it persists in food production, especially in poultry processing. Existing literature underestimates the environmental survivability of *C. jejuni* based on its microaerophilic sensitivity. However, recent reports on aerotolerant strains indicate that increased monitoring of this pathogen in poultry production is necessary. Therefore, the objective of this study was to characterize the aerotolerance of *C. jejuni* isolates that were isolated from commercial poultry processing plants. Twenty *C. jejuni* isolates previously isolated from commercial broiler processing plants were evaluated for aerotolerance and categorized into aero-sensitive, intermediate aero-tolerant, and hyper-aerotolerant groups. Isolates were cultured in BHI broth at 42°C under microaerophilic conditions for 18 hours. Working cultures of each isolate were prepared to approximately 5 log CFU/mL and incubated under aerobic conditions with shaking at 200 rpm at 42°C for 0, 12, and 24 h. Each isolate had three independent replicates. At each time point, samples were enumerated by plating onto *Campylobacter* agar base (Oxoid, CM0689) that was supplemented with antibiotics (Oxoid, SR0204E), and 5% laked horse blood. Isolates that failed to survive under aerobic incubation for 12 h were categorized as aero-sensitive, the isolates that survived for 12 h but did not survive until 24 h were categorized as intermediate aero-tolerant, and those survived for 24 h under aerobic incubation were categorized as hyper-aerotolerant. Samples incubated under microaerophilic conditions served as a control for each isolate. Out of 20 isolates, 8 isolates (40%) were aero-sensitive, 8 isolates (40%) were intermediate aero-tolerant, and 4 isolates (20%) were hyper aero-tolerant. Overall, more than half of the isolates demonstrated survivability in an oxygen-rich environment. These results indicate that intermediate aero-tolerant and hyper aerotolerant isolates are more prevalent in commercial broiler processing than previously expected which may be one of the major contributing factors for *Campylobacter* persistence.

Key Words: *Campylobacter*; Poultry processing; Aerotolerance

452P Withdrawn.

453P Survey of the U.S. Broiler industry regarding the implementation of *Campylobacter* performance standards on parts, and comminuted chicken products. Rafael Rivera^{1*}, Jinquan Wang¹, Harshavardhan Thippareddi¹, Manpreet Singh¹, ¹University of Georgia, Grayson, Georgia, United States.

Campylobacter is one of the most common foodborne pathogens associated with chicken and turkey products. In 2019, the Interagency Food Safety Analytics Collaboration (IFSAC) reported that over 80% of non-Dairy *Campylobacter* illnesses were attributed to chicken, other seafood (such as shellfish) and turkey. The USDA's Food Safety and Inspection Service (FSIS) announced introduced new performance standards for *Campylobacter* prevalence reduction in 2016 for parts and comminuted poultry. Enforcement of performance standards is temporarily suspended to test a new detection method to improve recovery. While implementation is delayed, there is time to evaluate overall industry action towards controlling *Campylobacter* in parts and comminuted chicken. A comprehensive on-line survey was conducted of key U.S. broiler industry stakeholders with the objective of assessing current *Campylobacter* performance standard status and intervention strategies to reduce in second and further processing operations. A total of 46 broiler processing operations with second and further processing processes responded. The association among answers were evaluated using lm function in version 4.0.2 (R Core Team, 2021). 90% of respondents indicated that they have established *Campylobacter* control programs for parts, ground, and other comminuted poultry. 70% of respondents indicated that they have an established company testing program for *Campylobacter* in second and further processing alongside FSIS sampling. The FSIS sampling program indicates that 46% are meeting parts performance standard while company data reports 64% meeting parts standards. 71% of respondents indicated that the establishment has validated interventions in second processing to reduce *Campylobacter*. There is no correlation between FSIS category and internal performance standard category from respondents. However, there is a significant correlation between FSIS sampling results and internal company data on whole carcass with a coefficient of 0.60 ($P < 0.01$), which suggested internal company data has a higher prevalence on whole carcass than FSIS results. FSIS category on both whole carcass and parts are based on the prevalence of *Campylobacter* detected in samples ($P < 0.05$). However, the category from company internal data showed that there is only correlation between the prevalence and internal category on parts ($P < 0.05$) but not on whole carcass. There is no correlation on peracetic acid concentration and *Campylobacter* prevalence. The survey results indicate that broiler companies are monitoring *Campylobacter* and adjusting interventions according to both FSIS and internal company results even

though the reporting and enforcement of performance standards are delayed.

Key Words: Campylobacter; Broiler; Food safety; Second and Further Processing; Qualitative survey

454P Molecular characterization of the interaction between *Campylobacter* and cecal microbiota in the presence or absence of different forms of casein *in vitro*. Elena Olson^{1*}, Dana Dittoe¹, Steven Ricke¹, ¹*University of Wisconsin-Madison, Verona, Wisconsin, United States.*

The ability of *Campylobacter* spp. to interact with the cecal microbiota makes understanding the metabolic exchanges occurring among these microorganisms important in identifying factors that impact *Campylobacter* colonization in the poultry gastrointestinal tract (GIT). The main objective of the current study was to screen three dietary modifications that utilized three distinct casein sources such as acid hydrolysate, enzymatic hydrolysate, and non-hydrolyzed casein as a representative model dietary proteins by naturally occurring cecal microbiota inoculated with *Campylobacter jejuni* *in vitro*. The secondary objective was to compare two different types of media, Bolton Enrichment Broth (BEB) representative of the commercial media used for *Campylobacter* growth and Anaerobic Dilution Solution (ADS) suited better for the natural environment of cecal microorganisms. Genomic DNA was extracted and amplified using custom dual-indexed primers and sequenced on an Illumina MiSeq. Sequencing data were analyzed in QIIME2-2021.4 with alpha and beta diversity being determined using Kruskal-Wallis and ANOSIM. The result showed that ADS supported a significantly more even and diverse microbial population compared to BEB. ANCOM analysis confirmed and indicated *Lactobacillus* as a numerically predominant genus in BEB followed by *Campylobacter* as also significant but numerically lower genus associated with BEB and ADS. All three different forms of casein in this study produced significantly different and better microbial evenness from the positive control ($P < 0.05$, $Q < 0.05$). ANCOM analysis identified *Lactococcus* and *Campylobacter* as significant genera associated with treatments and being numerically higher in the intact casein group. Significantly different microbial compositions were observed with time based on all metrics for alpha and beta diversities, where 0 h was significantly different from 24 h and 48 h. Interactions between media and modification, and time and modification were significant based on ANOVA and ADONIS analyses. The established metabolic pressures provided with different types of modifications and media showed that those metabolic characteristics are potentially due to competitive amino acid metabolism with lactic acid-producing bacteria. Thus, ADS should be considered to accurately reflect cecal microbial compositional changes due to amino acid supplementation in poultry feed. Additionally, the incubation period for treatments should not exceed 24 h as differences between 24 h and 48 h time points were not significant.

Key Words: amino acids; *Campylobacter*; metabolism; media; lactic acid-producing bacteria

455P Reduction of *Salmonella* and *Campylobacter* using photo-active antimicrobials. Andrea Urrutia^{1*}, Leticia Orellana Galindo¹, Alexandra Jackson¹, Marco Reina Antillon¹, Kenneth Macklin¹, Dianna Bourassa¹, ¹*Auburn University, Auburn, Alabama, United States.*

Salmonella and *Campylobacter* are two of the most common pathogens associated with poultry meat. Regulatory restrictions and consumer concerns have increased the need for natural antimicrobials and emerging novel technologies. In previous research, curcumin has been shown to be effective against *Salmonella*, but there are little available data for *Campylobacter*. The objective of this study was to determine the antimicrobial activity of three natural photo-active compounds curcumin (CUR), chlorophyllin (CH) and ascorbic acid (AA) alone and in combination followed by different LED light time exposure for the reduction of *Salmonella* and *Campylobacter*. Peroxyacetic acid (PAA), which has known antimicrobial activity, was also evaluated as a control. In 96 well plates, CUR, CH, and AA at concentrations of 100, 500, and 1,000 ppm, combinations of AA+CUR and AA+CH at 100 ppm each, PAA at 100, 200, and 400 ppm, and distilled water (DW) were evaluated. Each well was inoculated with 10^4 CFU/ml of *Salmonella* Typhimurium or *Campylobacter jejuni*. Plates were exposed to a LED light box (430 nm) for 0, 5, or 10 min. The treatments were serially diluted and plated on Brilliant Green Agar for *Salmonella* or Campy Cefex plates for *Campylobacter* and incubated aerobically for 24 h at 37°C or microaerobically for 48 h at 42°C, respectively. Data were analyzed for each antimicrobial by concentration and light exposure time using the GLM procedure with means separated by Tukeys HSD with significance at a P-value of ≤ 0.05 . There were no significant reductions in *Salmonella* following AA ($0.05 \log_{10}$ CFU/ml), CH ($0.05 \log_{10}$ CFU/ml), CUR ($0.06 \log_{10}$ CFU/ml), AA+CUR ($0.09 \log_{10}$ CFU/ml) or AA+CH ($0.12 \log_{10}$ CFU/ml) treatments. Likewise, there were no significant reductions in *Campylobacter* following AA ($0.03 \log_{10}$ CFU/ml), CH ($0.05 \log_{10}$ CFU/ml), CUR ($0.07 \log_{10}$ CFU/ml), AA+CUR ($0.09 \log_{10}$ CFU/ml) or AA+CH ($0.09 \log_{10}$ CFU/ml) between the DW control and the photoactive antimicrobials evaluated. No effect of photoactive antimicrobial concentrations or light exposure times were observed ($P \geq 0.8576$). Use of PAA at 400 ppm reduced *Salmonella* and *Campylobacter* to below detectible levels. At 200 ppm *Salmonella* counts were reduced by $2.42 \log_{10}$ CFU/ml and *Campylobacter* was reduced by $2.05 \log_{10}$ CFU/ml. At 100 ppm *Salmonella* was reduced by $1.54 \log_{10}$ CFU/ml and *Campylobacter* by $1.73 \log_{10}$ CFU/ml. These results indicate that CUR, CH, or AA alone and in combination were not effective as antimicrobials, particularly in comparison to the commonly used antimicrobial, PAA.

Key Words: Campylobacter; Salmonella; ascorbic acid; curcumin; chlorophyllin

456P Effect of a novel phytogetic water-soluble blend on intestinal *Salmonella* colonization and growth performance of broiler chicks. Paul Omaliko^{1*}, Emily Kimminau², Adedeji Adetunji¹, Tunde Ogundare¹, Yewande Fasina¹, ¹North Carolina A&T State University, Greensboro, North Carolina, United States, ²Land O' Lakes, Arden Hills, Minnesota, United States.

A study was conducted to evaluate the efficacy of Triplant, a novel phytogetic water soluble (WS) blend (Purina Animal Nutrition, MN) in reducing intestinal *Salmonella* colonization in broiler chicks. In a 14-day experiment, 212 day-old (Ross 708) male broiler chicks were obtained from a commercial hatchery, out of which 20 were confirmed free of our nalidixic acid-resistant *Salmonella* Enteritidis (SE) marker strain. Remaining chicks were weighed and randomly assigned to five dietary treatments in a completely randomized design (CRD). Treatment UC consisted of chicks fed unmedicated corn-soybean meal (SBM) basal diet without adding the Triplant blend into their water. Also, these chicks were not challenged with SE. Treatment IC consisted of chicks that were subjected to same treatment as UC chicks, but were additionally orally inoculated with SE (7.80×10^8 colony-forming units (CFU) SE / mL / chick) at 3 days of age. Treatments WS1, WS2, and WS3 consisted of chicks given a diet similar to that of UC and IC chicks, but their water was supplemented with Triplant blend at 0.075 g/L, 0.100 g/L, and 0.125 g/L, respectively, and chicks were also orally inoculated with SE as done with Treatment IC. Intestinal SE infection was confirmed and monitored throughout the experiment by enumerating cecal SE concentration on d 2, 5, and 11 post-challenge. Body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were also recorded to assess growth performance. Results showed that there were no differences ($P > 0.05$) among treatments for BW, BWG, FI, and FCR. However, on d 5 post-challenge (PC), chicks in Treatments WS2 (4.07 Log₁₀ CFU / g) and WS3 (3.01 Log₁₀ CFU / g) had lower ($P < 0.05$) cecal SE concentrations compared to IC chicks (5.09 Log₁₀ CFU / g). A similar trend was observed on d 11 PC. Also, chicks in Treatment UC remained free of SE throughout the experiment, indicating that care was taken to avoid cross-contamination among treatments. It was concluded that supplementation of Triplant blend in drinking water up to a concentration of 0.125 g/L reduced SE colonization in the ceca of broiler chicks without affecting growth performance.

Key Words: Salmonella Enteritidis; Growth performance; Broiler chicks; Cecal colonization

457P Conventional and free-range broilers: A look at the Enteric APEC. Smith¹, Jodi Delago¹, ¹Church & Dwight Inc, Waukesha, Wisconsin, United States.

Avian pathogenic *E. coli* (APEC) is a gram-negative bacterium with virulence that has led to significant economic losses in the poultry industry. These bacteria cause colibacillosis, a systemic disease which manifests in many ways such as airsacculitis, pericarditis, perihepatitis, and peritonitis. The objective of this study was to examine conventional and free-range (FR) broilers for differences in their enteric APEC diversity and levels. A total of 144 conventional and 120 FR broilers were harvested from three different companies (A, B, C) in Australia, from 1 to 6 weeks of age; conventional and FR birds were sampled from each company. Gastrointestinal tracts were collected to determine *E. coli* levels by plate counts. *E. coli* isolates were collected from each bird and subjected to an APEC multiplex PCR to determine pathogenicity. Screened pathogens were subjected to random amplified polymorphic DNA (RAPD) PCR to determine genetic diversity from which Shannon-Wiener diversity indices were generated, and a principal coordinate analysis was utilized to determine beta diversity. Count data was Log₁₀ transformed and analyzed by unpaired t-test to determine significant differences ($p < 0.05$). The total APEC level of conventional birds was significantly lower than FR broilers. FR broilers had a higher diversity of APEC strains, and APEC communities were more different between companies than between production types. In conclusion, though little research has been done to establish an understanding of APEC between these two broiler production types, the differences observed in this study could be attributed to differences in husbandry practices. Some previous research has shown that broiler stocking densities have affected the 16S fingerprint profiles of the bacterial community, and another study showed that diet played a role in shaping the microbial community within New Guinean passerine bird species. Additionally, duck rearing differences have been shown to impact the microbial diversity of ducks in aviary versus barn-raised environments. Though such studies may not parallel the current study in many ways, they provide unique insights that could potentially be of relevance to understanding the differences observed in this study.

Key Words: Broiler; Diversity; APEC; Free-range; Conventional

458P Detection of *Campylobacter* and *Salmonella* isolates from pullet and breeder farms of a commercial broiler company. Yagya Adhikari^{1*}, Matthew Bailey¹, James Krehling¹, Kaicie Chasteen¹, Luis Munoz¹, Cesar Escobar Lobo¹, Leticia Orellana Galindo¹, Pankaj Gaonkar¹, Steve Kitchens¹, Stuart Price¹, Jeff Buhr², Kenneth Macklin¹, ¹Auburn University, Auburn, Alabama, United States, ²USDA, Athens, Georgia, United States.

Assessment and control of foodborne pathogens like *Salmonella* and *Campylobacter* in the poultry industry is crucial for designing efficient food safety plans and programs to address emerging foodborne pathogens. A study was conducted with a commercial broiler company in

which two pullet farms (Farm 1 and 2 - 14 weeks) and four breeder farms (Farm 3, 4, 5, and 6 - between 35 to 45 weeks) were sampled to detect *Salmonella* and *Campylobacter*. With the findings, it will be possible to determine the risk factors of introducing these pathogens to the poultry complex. A total of 38 samples (boot swabs, insect traps, flypapers, feed samples) from inside and a total of 64 samples (insect traps, flypapers, soil samples, mouse intestine, unknown feces, puddles, litter pile, wild birds) from outside the pullet houses were collected for the isolation of *Salmonella* while 32 samples from inside and 35 samples from outside were used for isolating *Campylobacter*. Similarly, altogether 163 samples were collected for isolating *Campylobacter* and 177 samples for isolating *Salmonella* from inside and outside of four breeder houses. All the samples were analyzed with Molecular Detection System (MDS – 3M). From the two pullet farms, 5 MDS *Salmonella* positive samples (3 boot swabs, 1 soil sample, and 1 beetle sample), one boot swab from Farm 1 tested positive on culture for *Salmonella* while among 12 MDS *Campylobacter* positive samples (6 boot swabs, 4 soil samples, 1 unknown feces, and 1 mouse intestine), the mouse intestine from Farm 1 tested positive on culture for *Campylobacter*. From the four breeder farms, there were 58 MDS positive *Campylobacter* samples of which 4 samples (mouse intestine, boot swab, soil sample and fly paper inside) from Farm 4, and 2 puddle water samples from Farm 5, and 3 cow feces samples from Farm 6 tested positive for *Campylobacter* on culture. None of the samples tested positive for *Salmonella* in MDS. This study is survey-based and is more focused on identifying the potential risk factors for introducing *Salmonella* and *Campylobacter* to the complex. Thus, at this time, statistical analysis was not performed in this study. With these findings, it can be inferred that the surroundings of poultry houses include various potential risk factors that can potentially transmit foodborne pathogens *Salmonella* and *Campylobacter* into poultry houses, and hence, to the consumers. Strict biosecurity measures and implementing HACCP (Hazard Analysis Critical Control Points) plan along with periodic monitoring and surveillance for foodborne pathogens may be effective ways to avoid pathogens from entering poultry houses.

Key Words: breeder; pathogen; pullet; foodborne illness

459P Discovery of the intestinal microbiota that are associated with residual feed intake in broiler chickens. Jing Liu^{1*}, Qing Yang¹, Melanie Whitmore¹, Sydney Stewart¹, Kelsy Robinson², Glenn Zhang¹, ¹Oklahoma State University, Stillwater, Oklahoma, United States, ²USDA - ARS, Starkville, Mississippi, United States.

Feed accounts for 60-70% of the total cost in livestock and poultry production. Feed efficiency (FE) is thus critical in maintaining the profitability and sustainability while reducing the environmental impact. FE-associated bacteria in chickens have been reported, but the results are largely

inconsistent. The objectives of this study were to identify the gut bacteria that are linked to residual feed intake (RFI) and further compare FE-associated microbiota with earlier studies. To accomplish these, 72 male Cobb broilers were individually housed from day 7-35. Body weight and feed intake were recorded on a weekly basis and RFI calculated for each chicken. The luminal contents were collected from the ileum and cecum of 12 animals with the highest RFI and another 12 animals with the lowest RFI. Bacterial DNA was isolated from each broiler and subjected to 16S rRNA gene sequencing. Intestinal bacteria were classified using QIIME 2 and Deblur and subsequently compared between high and low RFI chickens. Our results showed that the microbiota compositions of high and low RFI chickens were significantly different in the ileum, but not the cecum. A number of bacteria were found to be differentially enriched in high and low RFI chickens. Moreover, *Oscillibacter* was consistently associated with highly efficient chickens, whereas *Subdoligranulum* was associated with lowly efficient chickens across two independent studies. These findings suggest a possibility of developing these gut bacteria as potential biomarkers for feed efficiency. They could also be explored to enhance feed efficiency.

Key Words: Gut microbiota; Broiler chickens; Feed efficiency; Residual Feed intake

460P Comparison of growth of *Campylobacter* spp. in a novel medium in primary containers incubated aerobically or anaerobically. Arthur Hinton^{1*}, Arturo Levican², Nelson Cox¹, ¹U. S. National Poultry Research Center, Athens, Georgia, United States, ²Pontificia Universidad Catolica de Valparaiso, Valparaiso, Chile.

The ability of cultures of *Campylobacter* spp. to grow in a new medium contained in primary containers incubated aerobically or anaerobically was examined. Ten ml of media composed of (g/L) beef extract, 50; tryptose, 10; soluble starch, 10; sodium bicarbonate, 5.0; sodium lactate, 3.0; agar, 0.5 was added to 25 ml, 12.5 cm² culture flasks. The media was inoculated with 10⁴ cfu/ml of *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter jejuni*, or *Campylobacter lari*. The flasks were then closed with plug-sealed caps, vented caps, or vented caps covered with Parafilm laboratory film. The cultures were incubated at 37C for 48 h aerobically under a normal atmosphere or anaerobically in a Coy Anaerobic Chamber. After incubation, the number of cfu/ml of *Campylobacter* spp. in the media was enumerated on a *Campylobacter* selective agar composed of Blood Agar Base #2 supplemented with 7.0% horse blood and Blaser-Wang antibiotic mixture. The inoculated, selective agar was incubated microaerobically at 37C for 48 h, and cfu were counted. Significant differences in data were determined using GraphPad InStat statistical software. Results indicated that there were significant differences ($p \geq 0.05$) in the number of each *Campylobacter* spp. recovered from vented flasks incubated aerobically or anaerobically.

No *Campylobacter* spp. were recovered from vented flasks incubated aerobically, while between 4.44 and 5.67 log cfu/ml were recovered from vented flask incubated anaerobically. Conversely, there was no significant differences in the number of *Campylobacter* spp. recovered from Parafilm-sealed or plug-sealed flasks incubated aerobically or anaerobically. Between 8.34 and 8.68 log cfu/ml were recovered from Parafilm-sealed flask incubated aerobically while between 7.36 and 8.10 log cfu/ml were recovered from Parafilm-sealed flasks incubated anaerobically. Furthermore, between 8.17 and 8.46 log cfu/ml were recovered from plug-sealed flasks incubated aerobically, while between 7.84 and 8.69 log cfu/ml were recovered from the flasks incubated anaerobically. Findings indicate that *Campylobacter* spp. can grow in the new medium in primary containers that can maintain an atmosphere that supports the growth of the pathogen. Other research has indicated that the carbon dioxide concentration of the atmosphere in the containers is critical supporting *Campylobacter* growth. The new medium will simplify procedures for growing *Campylobacter* because artificial atmospheres will not have to be generated to grow the bacterium.

Key Words: *Campylobacter*; medium; aerobic; anaerobic; atmosphere

461P Microbiota modulated bile acid metabolites influence *Campylobacter jejuni* in vitro growth. Ying Fu^{1*}, Hong Wang¹, Tahrir Alenezi¹, Ayidh Almansour¹, Xiaolun Sun¹, ¹University of Arkansas, Fayetteville, Arkansas, United States.

Campylobacter jejuni asymptotically colonizes in the gut of chickens, but it is one of the pathogenic bacteria for many other animals with limited mechanism understanding. One of the differences between these animals is their various bile acid compositions. The bile acids in *C. jejuni*-resistant mice are dominant with cholic acids (CA), while chenodeoxycholic acid (CDCA) is the main bile acid in *C. jejuni*-susceptible chickens. The data we presented previously shown that CDCA in diet changed the mice susceptibility to *C. jejuni* infection. The objective of this study was to unravel the underlying mechanism of how bile acids influenced *C. jejuni* in vitro growth. In this study, *C. jejuni* 81-176 was inoculated in Brain Heart Infusion (BHI) broth in the presence of CA/ CDCA at physiological level of 1.5 mM alone, coculture with CDCA- or basal diets-modulated microbiota that collected from mice small intestine, cecum, or colon. *C. jejuni* was also cultured in filtered supernatant from mouse ileal microbiota 24 hr precultured with 1.5mM CDCA or CA (MB-super, CDCA-MB-super, or CA-MB-super). After 24 h cocultured in anaerobic condition mimicking mouse intestine, *C. jejuni* was enumerated by PBS serial dilution and plating. Interestingly, both CDCA and CA at the level of 1.5 mM reduced *C. jejuni* growth in vitro. Similarly, the small intestinal microbiota from mice fed basal or CDCA diet also

reduced *C. jejuni* growth, same as the diets-modulated microbiota in cecum and colon. These results suggest that CDCA, CA or microbiota alone reduces *C. jejuni* growth. Remarkably, CDCA in the presence of CDCA or CA-modulated SI microbiota increased the *C. jejuni* growth significantly, while CA plus CDCA or CA-modulated SI microbiota reduced the *C. jejuni* growth significantly. These results suggest that CDCA metabolized by microbiota promotes *C. jejuni* growth, while CA metabolized by microbiota reduces the pathogen growth. Notably, CDCA-MB-super increased *C. jejuni* growth compared to MB-super, whereas CA-MB-super reduced *C. jejuni* growth compared to MB-super. In conclusion, CDCA or CA derivatives from microbiota metabolism might be the driving force to alter *C. jejuni* growth, suggesting potential role of bile acid metabolites on the bacterial animal infection.

Key Words: microbiota; metabolomics; Bile acid composition; *Campylobacter jejuni* susceptibility; coculture

462P Microbial communities and antimicrobial resistant genes in small-scale poultry farms in Tennessee. Agnes Kilonzo-Nthenge^{1*}, Tobenna Anume¹, ¹Tennessee State University, Nashville, Tennessee, United States.

Antimicrobial usage in animal production contributes to the development of antimicrobial resistance in bacteria. Antimicrobial resistance is progressively a major threat to public health, making it difficult to treat infections caused by microorganisms that are no longer susceptible to commonly used antibiotics. The present study characterized microbial communities, antibiotic resistant genes, and virulence factors in poultry farms in Tennessee. Soil, water, and fresh feces were collected from three farms in triplicates. The genomic DNA was extracted from all samples and subjected to metagenomic analysis. Unassembled sequencing reads were directly analyzed to reveal associated microbial community composition. Our results displayed a diversity of microbial communities in soil, feces, and water. *Proteobacteria* (42.3%) and *Actinobacteria* (26.1%) in soil were significantly more ($p < 0.05$) than *Acidobacteria* (8.5%), *Planctomycetes* (5.2%). Whereas in feces, *Firmicutes* (36.62%), *Proteobacteria* (21.50%), and *Bacteroidetes* (15.76%) were also prevalent. However, *Cyanobacteria* (81.92%) significantly ($p < 0.05$) displayed the highest relative abundance among bacterial species present in poultry farms. Relative abundance of virulence genes functionally in poultry farms were demonstrated in *Salmonella* Typhimurium (0.3%), *Enterococcus faecalis* (10.1%), *Staphylococcus lentus* (5.7%), *Streptococcus pyogenes* (2.9%), *Bacteroides fragilis* (42.3%), *Escherichia coli* (4.2%), *Klebsiella pneumoniae* (11.7%), *Proteus mirabilis* (9.02%), *Pseudomonas aeruginosa* (2.05%), and *Vibrio cholerae* (1.1%). *Proteus mirabilis* GENE tnpA, a virulence gene was only displayed in water samples.

Antimicrobial resistant genes including aminoglycoside (aadA, aph), beta-lactam-resistance (cfxA, pbp, blaOXA, blaTEM), macrolide (InuC, ermF), repressor-for-mdr-efflux-pump (crp, emrR), tetracycline (tet40, tet44, tetO, tetQ, tetW, tetX), and trimethoprim (dfrD) were characterized in feces, water, and soil. Antibiotic resistant genes for tetracycline in feces (52.2%) and water (50.2%) were significantly ($p < 0.05$) more abundant as compared to soil (0%). Resistant genes to aminoglycoside (12.5%), trimethoprim (17.1%), macrolide (8.7%), and sulphonamide sul 2 (4.9%) were only displayed in feces and not in water and soil. Notably, resistance genes for beta-lactam were significantly ($p < 0.05$) the most abundant in all farms. Overall, the findings of this study demonstrate that antibiotic resistant bacteria and virulence genes are prevalent in poultry farms, which has serious implications to food safety. Antimicrobial resistance in both pathogenic and commensal bacteria in poultry farms may spread to humans, hence a great public health concern.

Key Words: Antimicrobial resistance; poultry farms; virulent genes; pathogenic; commensal bacteria

463P Exploring *Salmonella* populations over time within a broiler complex using CRISPR typing. Zachary Zawada^{1*}, Jodi Delago¹, Shannon Burasco¹, Alexandra Smith¹, ¹Arm & Hammer Animal and Food Production, Milwaukee, Wisconsin, United States.

Salmonella is one of the leading causes of bacterial foodborne illness in the US. Therefore, understanding the prevalence of *Salmonella* and how the serovars that are present change is important for producers in the poultry industry. To examine this, broiler farms that have historically shown to have had a high prevalence of *Salmonella* were targeted for this study. Five farms were selected with two houses from each farm being followed over five production cycles. The farms were sampled at 10 to 18 days of age (field samples) as well as at market age. Ceca samples were collected from each farm during each production cycle (6 birds per house) with market aged birds being sampled post harvest at the plant. *Salmonella* was enumerated through a mini-MPN method to determine *Salmonella* levels and CRISPR-2 typing was used to determine the serovars of the *Salmonella* isolates recovered. A t-test comparing the *Salmonella* levels across the first five production cycles showed a small but significant difference ($p < 0.05$) with the average *Salmonella* levels lower in the market aged birds (0.97 ± 1.263 Log CFU/g) compared to the field birds (1.23 ± 1.507 Log CFU/g). CRISPR typing results found that through four production cycles the *Salmonella* population had three to seven different serovars recovered per cycle. *Salmonella* Infantis and *Salmonella* Kentucky were the only two serovars present through all production cycles for both the field and the market aged birds and were the most prevalent isolates recovered. *Salmonella* Typhimurium was detected in every

cycle across both groups except for cycle two and cycle three for the field samples. A few serovars were only recovered periodically with *Salmonella* Ouakam being recovered in the first cycle from the field samples and *Salmonella* Hadar only being recovered in the second cycle from the market aged birds. *Salmonella* Mbandaka was recovered at both the first and the fourth cycle of the field samples while *Salmonella* Enteritidis was only detected in the second and third cycles with neither being recovered in the market aged birds. A few unknown serovars were recovered during the first and fourth cycles of the field samples and the first cycle of the market aged birds. In general, the populations present in the field samples and at market age were different from one another with *Salmonella* Infantis and *Salmonella* Kentucky being the only serovars consistently present in both groups during all of the production cycles.

Key Words: *Salmonella*; CRISPR Typing

464P Effect of *Bacillus amyloliquefaciens* supplementation on reduction of Enteric Pathogens in Broiler 16S rRNA Small Intestinal Microbiome Datasets. Joshua Lefler¹, Brian Dirks^{1*}, James Gaffney¹, Kayla Calapa¹, Andrew Izzo¹, Mallory Embree¹, ¹Native Microbials, Norristown, Pennsylvania, United States.

Broiler pathogens contribute to billions of dollars of lost production annually in the broiler industry. Pathogens like *Clostridium perfringens*, *Escherichia coli* and *Mycoplasma* spp., or foodborne pathogens, such as *Salmonella enterica*, contribute to revenue loss by increasing flock mortality and contaminating poultry meat. As such, developing methods to monitor and reduce the levels of these pathogens during broiler production is of global importance. Here, we report the effect of a strain of *Bacillus amyloliquefaciens* (Avis ProtectTM, Native Microbials, Inc.), originally isolated from the gastrointestinal (GI) tract of a healthy broiler chicken, on the abundance of pathogens in the GI tract using 16S rRNA sequencing. Over 4000 samples were collected from 11 sites across the United States for microbiome analysis. Microbiome analysis was conducted using Usearch (v11.0) for sequence processing, OTU generation and subsequent analysis. Sequences were cross referenced with an in-house database of broiler pathogens to determine the abundance of each pathogen of interest. A sequence was considered a match if it had >99% sequence identity to the pathogen of interest. A t-test was used to compare abundances between control birds and birds treated with *B. amyloliquefaciens*. Results showed a decrease in the relative abundance for a variety of pathogens ($P < 0.05$): 45% for *C. perfringens*, 41% for *Enterococcus cecorum*, 35% for *Staphylococcus gallinarum*, 51% for *Campylobacter jejuni*, and 54% for *S. enterica* in birds treated with *B. amyloliquefaciens*. Further, the microbiome of treated birds exhibited a lower level of alpha diversity when compared to control birds, indicative of a streamlined, efficient intestinal microbiome. These

results demonstrate the efficacy of using 16S rRNA microbial datasets for pathogen surveillance, as well as the apparent efficacy of this *B. amyloliquefaciens* strain to reduce the abundance of pathogens in the broiler GI tract.

Key Words: Broiler; Microbiome; Bacillus; Pathogens; 16S rRNA Sequencing

465P High-pressure processing reduces *Salmonella* and *Campylobacter* in chicken by-products (gizzards, livers and hearts). Sasikala Vaddu^{1*}, Manpreet Singh¹, Harshavardhan Thippareddi¹, ¹University of Georgia, Athens, Georgia, United States.

High-pressure processing (HPP) is a non-thermal technique and can be used to inactivate foodborne pathogens and spoilage organisms in a variety of food matrices. Chicken livers have been implicated in salmonellosis outbreaks in the U.S. The objective of this study was to evaluate the efficacy of HPP against *Salmonella* and *Campylobacter* in various chicken by-products (gizzards, livers and hearts). Fresh chicken gizzards, livers, and hearts (0.45 kg each) were obtained and inoculated with a cocktail of nalidixic acid-resistant *Salmonella* Typhimurium (ST^N; ca. 6 log₁₀ CFU/mL) and gentamicin resistant *Campylobacter coli* (CC^G; ca. 5 log₁₀ CFU/mL). The inoculum was allowed to attach for 15 min, vacuum-packed in high barrier bags, and cooled to 4°C. HPP was performed under intense pressure (about 85 MPa) at process temperatures (<19°C) for variable lengths of time (1 s, 1.25, 2.5 and 5 min) and pressure was released after each treatment time. HPP treated samples were rinsed in chilled buffered peptone water (100 mL), serially diluted in peptone water, and spread plated on Brilliant Green Sulfa agar supplemented with nalidixic acid (200 ppm) for enumerating *Salmonella* and on Campy Cefex agar containing gentamicin (200 ppm) to enumerate *Campylobacter*. Three independent replications (n=3) were performed using freshly obtained chicken by-products on three different days. Data were analyzed using one-way ANOVA in the GLM of SAS 9.4 (2004; SAS Institute Inc., Cary, NC). Tukey's test was used to separate the means of the microbial reductions (log₁₀ CFU/mL) and significant difference at P≤0.05 was used. HPP resulted in ST^N reductions (p≤0.05) of 5.06, 5.18 and 3.81 log CFU/mL within 5 min in gizzard, heart and liver. CC^G population reductions (p≤0.05) of >5.00 log CFU/mL were observed within 1 s of HPP treatment in all the chicken by-products. Shorter HPP treatment times resulted in lower ST^N and CC^G population reductions. *Campylobacter* was more sensitive to HPP treatment compared to *Salmonella* in chicken by-products. HPP of chicken by-products (gizzards, livers and hearts) can improve microbiological safety of the products by reducing *Salmonella* and *Campylobacter* populations.

Key Words: Campylobacter; Salmonella; high-pressure processing; chicken by-products; livers

466P Early colonizing bacteria of turkey poult effect microbial succession. Josh Rehbecker^{1*}, Enid McKinley¹, Jodi Delago¹, Theresia Lavergne¹, Alexandra Smith¹, ¹Arm & Hammer, Waukesha, Wisconsin, United States.

Three replicate trials were run to determine the consistency of the early colonizing bacteria found at day of hatch (DOH) and its impact on the succession of the microbiome over the life of poult. Trials commenced in February, August, and November and terminated after 49 days. For each trial 22 poult were housed in each of 24 pens. Poults were sacrificed at hatch, day 7, 14, 21, 28, and 35. At each time point mucosal associated communities were collected for DNA extraction and Illumina sequencing of the V4 region of the 16s rDNA gene. Sequences were quality filtered, denoised, and taxonomy was assigned at the genus level. Resulting data were log transformed, centered by genus, and analyzed by linear constrained ordination. When the three trials were constrained by trial with all timepoints included, the microbiota were significantly different in all three trials (P < 0.05) accounting for 5.2% of the variation. When the three trials were constrained by age, neglecting the effect of trial, the microbiota at each age were different than all other ages (P < 0.05) with age accounting for 17.3% of the variability. The greater explained variation by age indicated that each age should be evaluated independently for differences between trials. Microbial populations at DOH for trial 1 and trial 3 were significantly different from each other and trial 2 (P < 0.05). For weeks 1 through 5 the microbiota from each trial at any time were different (P > 0.05) from the microbiota for all other trials, except for trial 1 at week 1 and week 3 which tended to be different (0.05 < P < 0.10). The DOH microbiota for trial 1 were comprised primarily of *Clostridium*, while trials 2 and 3 primarily consisted of *Escherichia* with trial 2 having a lesser proportion of *Clostridium* and trial 3 a lesser proportion of *Enterococcus*. At week 1 *Lactobacillus* was predominant in trial 1 and 3 with trial 1 having a lesser proportion of *Arthromitus* and trial 3 containing *Escherichia*. At week 1 microbiota from trial 2 consisted of a diverse proportion of 6 genera. Starting at week 2, *Lactobacillus* became the most predominant genus in all three trials. The microbiota of trial 1 had lesser proportions of *Bacillus* at week 2 followed by *Streptococcus* in weeks 3 through 5. During weeks 2 through 5 *Megamonas* and *Helicobacter* were also prevalent in the microbiota of both trials 2 and 3 but *Megamonas* was more predominant in trial 2 and *Helicobacter* in trial 3. The differences in the microbiota of these three trials highlight the variability of poult sourced from the same hatchery and reared in the same environment. This data indicates that early colonizing bacteria effect the microbial succession which may impact performance.

Key Words: microbiome; turkey; poult

467P Profiling intestinal proliferative cells in the small intestine of broiler chickens via in situ hybridization

during the peri-hatch period. Sara Cloft^{1*}, Zehava Uni², Eric Wong¹, ¹Virginia Tech, Blacksburg, Virginia, United States, ²The Hebrew University of Jerusalem, Rehovot, Israel.

Chickens are a precocial species that hatch ready to consume food without requiring parental intervention. This is accomplished through an activation and maturation process of the small intestine which begins around embryonic day (e) 17 when the embryo consumes amnion fluid. A defining feature of the mature small intestine are intestinal crypts populated by stem cells producing replacement cells to maintain the absorptive villus surface area. The embryonic intestinal crypt is rudimentary and cells along the villi are capable of proliferation; however, by 7 days post-hatch (d) the crypts are fully functional and proliferative activity along the villi is minimal. The objective of this study was to profile the changes of stem cell and proliferative genes: Olfactomedin 4 (Olfm4), Leucine-rich repeat containing G protein-coupled receptor 5 (Lgr5), and marker of proliferation Ki67 during the peri-hatch period. Six embryos or chicks were randomly selected on e17, e20, d1, d3, and d7 for small intestinal collection. All 3 intestinal segments were collected and in situ hybridization (ISH; n=3/timepoint) and quantitative PCR (n=6) was conducted for each gene. Log transformed data were analyzed using a factorial ANOVA considering segment and timepoint. During the peri-hatch period villi from all intestinal segments exhibited characteristic maturation from small pear-shaped to long narrow structures. Similarly, crypts deepened and began multiplication crypt fission by 1d. All genes had significant interactions of segment and timepoint. Expression of the 2 stem cell marker genes Olfm4 and Lgr5 differed. Olfm4 mRNA increased post-hatch while Lgr5 decreased post-hatch. The proliferation marker Ki67 mRNA did not change with time but generally ileal mRNA was the greatest. The ISH is consistent with the qPCR results. Olfm4 was only ever seen in the crypts and increased along with age. In contrast Lgr5 was expressed in the crypt and the villi in the embryonic periods but become restricted in the intestinal crypt during the post-hatch period. Ki67 was expectedly expressed throughout the intestine during the embryonic periods. Though as time went on the expression pattern became restricted to the crypt and the central lacteal of the villi. The changes of proliferative cells shown in this study are consistent with previous research showing that the small intestine develops intestinal crypts populated with stem cells that produce replacement cells and villi composed of non-proliferative functionally differentiated cell types during the first week post-hatch.

Key Words: In situ hybridization; Lgr5; Olfm4; Peri-hatch; Ki67

468P The impact of semen insemination on turkey hen sperm storage tubule transcriptome dynamics. Kristen

Brady^{1*}, Julie Long¹, ¹USDA-ARS, Beltsville, Maryland, United States.

Sperm storage tubules (SST) are structures of the uterovaginal junction of the oviduct that allow poultry to store sperm for extended periods. Aspects of SST function remain unknown, including the role of the local immune environment, sperm maintenance mechanisms, and tissue remodeling processes. A global approach was taken to characterize transcriptome changes throughout the duration of fertility following semen insemination. SST were isolated from virgin, sham inseminated, and semen inseminated (2 pre-lay and 1 post-lay insemination) turkey hens at day 1, 30, and 90 post-insemination (n=3/group), using laser capture microdissection techniques. Extracted RNA was sequenced on an Illumina HiSeq platform. Bioinformatic and gene ontology analysis were performed using CLC Genomics software and DAVID, respectively. RNA sequencing gene expression results were confirmed through RT-qPCR, with data analyzed by a two-way ANOVA using the mixed models procedure of SAS. RT-qPCR determine mRNA levels were consistent with the gene expression levels and trends obtained through RNA sequencing. A total of 798 differentially expressed genes (DEGs) were identified due to group, with 160 DEGs at D1, 471 DEGs at D30, and 167 DEGs at D90. DEGs were enriched with non-coding RNAs (ncRNAs) across all three timepoints, with 13.4% of the total DEGs classified as ncRNAs. A total of 65 coding genes were differentially expressed at two or more of the timepoints, and were functionally related to cell motility, extracellular matrix organization, and lipid metabolism pathways. Functional annotation of the timepoint unique coding genes showed enrichment at D1 for innate immune response, chondrocyte differentiation, and thyroid hormone metabolism pathways, with semen inseminated samples showing upregulation of cytokine response genes and downregulation of thyroid hormone metabolism genes. At D30, virgin and sham inseminated samples showed upregulation of axonogenesis and dendrite formation while semen inseminated samples showed upregulation of ATP and carbohydrate derivative metabolism. At D90, virgin and sham inseminated samples showed upregulation of ion homeostasis and cell death pathways when compared to semen inseminated samples. Semen insemination altered the SST transcriptome at D1, D30, and D90 post-insemination, with gene expression shifted towards innate immune pathways at early timepoints and sperm preservation mechanisms at later timepoints. With the large proportion of ncRNAs identified in this study, further studies are needed characterize the role of ncRNAs, potentially as regulators of gene expression essential for SST function in response to insemination.

Key Words: fertility; turkey; RNA sequencing; artificial insemination; sperm storage tubules

469P Hypothalamo-pituitary-gonadal axis receptor expression changes during follicle development in RBC1

and E line turkey hens. Diane Hildenberger^{1*}, Julie Long¹, Kristen Brady¹, ¹USDA-ARS, Beltsville, Maryland, United States.

Egg production rate depends on precise regulation of signals to and from developing ovarian follicles, including gonadotropin stimulation and steroid hormone feedback. Follicle development stages are characterized by physiological and morphological changes ultimately leading to follicle ovulation, with early stages comprised of slow growth from white yolk incorporation and later stages comprised of rapid growth from yellow yolk incorporation. Follicle selection occurs when a single prehierarchical follicle enters the preovulatory phase, which is marked by changes in cell signaling that contribute to follicle maturation, survival, and rapid growth. Hypothalamo-pituitary-gonadal (HPG) axis genes regulate follicle development and steroid production dynamics. This study compared ovarian morphological parameters and HPG axis gonadotropin and steroid hormone receptor mRNA levels in large white follicles (LWF, 3-5mm), small yellow follicles (SYF, 6-8mm), and the most recently selected follicle (SF, 11-13mm) to examine prehierarchical follicle growth and selection differences between turkey hens specifically bred for high egg production (E line) and a random bred control (RBC1) (n=5 hens/group) during peak egg production. Total RNA was extracted with QIAGEN RNeasy kits, and transcription levels were measured by reverse transcription quantitative PCR. Normalized mRNA levels between E line and RBC1 groups were analyzed using the PROC mixed protocol in SAS. Body weight and F1 follicle weight differed between E line and RBC1 hens, with RBC1 weights higher than E line weights ($p<0.05$), but differences were not seen in ovary weight, prehierarchical follicle weights, and number of preovulatory follicles. Luteinizing hormone receptor mRNA levels were higher in RBC1 LWF, whereas follicle stimulating hormone receptor levels were higher in E line SYF ($p<0.05$). Estrogen receptor 1 mRNA levels were higher in RBC1 LWF compared to E line hens ($p<0.01$). Progesterone receptor levels were higher in RBC1 LWF but higher in E line SF ($p<0.05$). No differences in mRNA levels were observed in estrogen receptor 2 or androgen receptor in any follicle stage. Differences in mRNA levels for gonadotropin and steroid hormone receptors could indicate key disparities in gonadotropin responsiveness and/or steroid hormone feedback between RBC1 and E line follicles. Differences in HPG axis receptor mRNA levels may explain increased egg production in E line hens via regulation of steroidogenesis and folliculogenesis. Further studies are warranted to determine the downstream effects of HPG axis receptor expression differences in RBC1 and E line turkeys.

Key Words: gene expression; egg production; turkey; folliculogenesis; follicle selection

470P Effects of thyroxine on genes within the somatotrophic axis in avian muscle cells. Lauren Vaccaro^{1*},

Addison Smith¹, Laura Ellestad¹, ¹University of Georgia, Athens, Georgia, United States.

Crosstalk between the thyrotropic and somatotrophic endocrine axes regulates growth and metabolism and could involve thyroid hormone (TH) modulation of insulin-like growth factor (IGF) and IGF binding protein (IGFBPs) expression. Previously, triiodothyronine (T_3) treatments at biologically relevant levels were found to alter *IGFBP* mRNA expression in Quail Muscle Clone 7 (QM7) cells *in vitro*. This suggests that T_3 -induced IGFBP activity could restrict or induce local muscle tissue growth and the precursor to T_3 , thyroxine (T_4), may regulate IGFBP expression. The objective of this study was to determine effects of T_4 on somatotrophic and thyrotrophic gene expression in QM7 cells. Undifferentiated and differentiated QM7s were treated with 0, 1, 5, or 25 ng/mL T_4 for 0.5, 6, or 24 hours, and mRNA expression was determined by RT-qPCR ($n=3$). Data were analyzed by two-way ANOVA, and *post hoc* means comparisons were made with the test of least significant difference when ANOVA indicated significance ($P\leq 0.05$). In undifferentiated cells, 5 and 25 ng/mL T_4 increased growth hormone receptor (*GHR*) expression at 6 hours ($P\leq 0.05$). By 24 hours, only 25 ng/mL T_4 had the same effect. At 6 hours, *IGFBP5* mRNA levels were greater in cells treated with 5 and 25 ng/mL T_4 ($P\leq 0.05$), and all three doses significantly increased *IGFBP5* expression at 24 hours ($P\leq 0.05$). Thyroid hormone receptor alpha (*THRA*), IGF receptor 1 (*IGFR1*), *IGFBP2*, and *IGFBP3* expression were not affected by T_4 treatment ($P>0.05$). In differentiated cells at 6 hours, *IGFBP7* expression was nearly significantly lower in cells treated with 5 ng/mL T_4 than those without T_4 ($P=0.0642$). A main effect of T_4 concentration also approached significance for *IGF2* in differentiated cells, with 25 ng/mL decreasing *IGF2* expression ($P=0.0515$). These data suggest that T_4 may regulate somatotrophic gene expression in muscle cells, possibly altering cellular proliferation. Local muscle tissue growth could be stimulated or inhibited by circulating T_4 via control of IGFBP5 activity. Heightened *GHR* may increase the sensitivity of muscle cells to GH signaling, leading to increased local paracrine IGF signaling induced by GH, thus impacting proliferation. Differentiated muscle cells appear less responsive to T_4 than undifferentiated muscle cells. Previously, we found that altered somatotrophic gene expression by T_3 was not cell-status specific. This suggests that undifferentiated muscle cells are more effective at converting T_4 to T_3 , possibly through increased deiodinase activity as compared to differentiated cells. Deiodinase mRNA levels will be measured forthwith to test the hypothesis that differences in their expression exists between undifferentiated and differentiated cells.

Key Words: growth hormone receptor; thyroid hormone; insulin-like growth factor binding proteins; avian muscle

471P Effects of delayed post-hatch feeding stress on later life enteric nervous activities and intestinal functions in broiler chickens under normal and stress conditions. Lydia Schlitzkus¹, Liang-en Yu¹, Peter Mann¹, Yihang Li^{1*}, ¹University of Delaware, Newark, Delaware, United States.

Early life stress alters the developmental trajectory of intestine, leading to functional disorders in later life. The enteric nervous system (ENS) that coordinates functions of the intestine is sensitive to stress and has been overlooked in agricultural animal's health. In the present study, we evaluated the long term consequences of the post-hatch delayed feeding stress on intestinal barrier function, nutrient transport, and ENS activities. Total of 108 Ross 308 eggs were hatched and allotted to a delayed-feeding group (DF, no feed until 72h post-hatching) or a normal-feeding group (NF, immediate access to feed). Both groups were raised under the same condition until d42 of age. A secondary stress challenge of 24h-starvation was conducted at d43 of age. Growth performance was recorded. Ileal functions were evaluated on Ussing chambers, including barrier function indicated by transepithelial resistance (TER) and FITC-dextran (FD4) permeability; nutrient transport function indicated by short circuit current (*I*_{sc}) changes in response to nutrients application; and ENS activities indicated by *I*_{sc} change after veratridine stimulation. Ileal brush boarder membrane (BBM) transporters for glucose, glutamate, and dipeptide (SGLT1, EAAT3, and PepT1, respectively) were measured by western blotting. Gene expressions of enzymes and receptors of neurotransmitters were measured by RT-PCR. Student's t-test was performed for statistical analysis. Our results showed a compensatory growth in DF birds. At d42 of age, the ileum of DF birds exhibited upregulated ($P < 0.1$) electrogenic transport of dipeptide and lysine, as well as increased BBM nutrient transporters protein level, compared with NF control. Gene expression of ileal choline acetyltransferase (ChAT) was significantly ($P < 0.05$) upregulated in DF birds. Veratridine activated neural responses were significantly higher ($P < 0.05$) in the ileum of DF birds. Ileal TER was significantly ($P < 0.05$) reduced in DF birds, while FD4 permeability was increased compared with NF control. The upregulated nutrient absorption via transcellular and potential paracellular routes may support compensatory growth in DF birds. However, after being exposed to the secondary stress, the reduction of TER and increase in FD4 were exacerbated in DF birds ($P < 0.05$), while the electrogenic nutrients transport and BBM transporters were no longer different between DF and NF birds. In summary, our results indicated that early life stress triggered long lasting leaky gut, upregulated nutrient transport, and hyperactive ENS in broilers. Such alterations may be beneficial to the compensatory growth in an optimal environment, but be deleterious for animal's health under stressful conditions.

Key Words: Gut health; Intestinal barrier; Delayed feeding; Enteric nerves; Intestinal nutrient transport

472P Chicken yolk sac tissue as a source of endocrine factors during early embryogenesis. Brett Marshall^{1*}, Grant Bennett¹, Eric Wong³, Israel Rozenboim², Zehava Uni², Laura Ellestad¹, ¹University of Georgia, Winterville, Georgia, United States, ²The Hebrew University of Jerusalem, Rehovot, Israel, ³Virginia Tech, Blacksburg, Virginia, United States.

For at least the first two weeks of chicken embryogenesis, a substantial increase in size occurs prior to the development and maturation of endocrine systems that regulate growth. Yolk contains nutrients and growth factors that can be used for embryonic development, with the yolk sac tissue (YST) serving as the intermediary between the yolk and the chick itself. Whether YST serves solely as a passive intermediary to regulate access to these nutrients and growth factors or actively contributes to embryo growth by producing hormones prior to development of endocrine tissues is unclear. Therefore, the objective of this study was to discern if YST participates in early endocrine regulation of growth through production of hormones and other factors within the somatotrophic and thyrotrophic axes. Four broiler embryos were harvested on embryonic (E) days 3, 6, 9, and 12 for collection of YST (n=4). Total RNA was isolated, and RT-qPCR was used to measure expression of hormones, receptors, binding proteins, and enzymes within each axis. A one-way ANOVA followed by Fisher's test of least significant difference was conducted. Within the somatotrophic axis, growth hormone receptor expression increased from E3 to E6 ($p \leq 0.05$), and while insulin-like growth factor (IGF) 1 was not detected, *IGF2* was expressed highly and numerically greatest on E3 ($P=0.096$). All IGF binding proteins (IGFBPs) were expressed in YST, with most exhibiting increases or decreases throughout development. *IGFBP1* increased steadily between E3 and E9 ($P \leq 0.05$), *IGFBP5* and *IGFBP7* increased from E3 to E6 ($p \leq 0.05$), and an increase in *IGFBP3* between E3 and E6 approached significance ($P=0.0579$); all remained elevated through E12. *IGFBP4* exhibited a transient increase in expression on E6 compared to the other ages ($P \leq 0.05$). In stark contrast, *IGFBP2* levels were highest in YST on E3, dropped through E9 ($p \leq 0.05$), and remained low on E12. Within the thyrotrophic axis, thyroid hormone (TH) converting enzyme deiodinase (DIO) 1 was lowest in the YST on E3 and increased and leveled off on E6 ($p \leq 0.05$). In contrast, *DIO2* was highest on E3, dropped dramatically on E6, and continued to decline through E9 ($p \leq 0.05$). Overall, these results suggest the YST produces IGF2 and plays a role in regulating its availability through dynamic expression of IGFBPs. Additionally, as *DIO2* is critical for converting TH to its more bioactive form, the YST likely regulates embryo utilization of TH stores within the yolk. Together, these results suggest that the YST is an active participant in the endocrine regulation of early embryonic growth and metabolism.

Key Words: growth; insulin-like growth factor binding proteins; somatotrophic axis; thyrotrophic axis; thyroid hormone deiodinases

473P Early non-destructive identification of breeding laying hens egg fertilization status through egg odor analysis. Xiaojuan(Judy) Song^{1*}, Christopher Heist¹, Olga Kemenova¹, Douglas F. Britton¹, Jeanna L. Wilson², ¹Georgia Tech Research Institute, Atlanta, Georgia, United States, ²University of Georgia, Athens, Georgia, United States.

Fertility and hatchability rates continue to dwindle from year to year resulting in losses of millions of dollars annually and remains of a great economic concern to poultry farm owners globally. Therefore, it is critical to determine fertility and viability of chicken eggs prior to incubation. However, early and non-destructive identification of fertility is a difficult task in the process of breeding laying hens. It has been shown that volatile organic compounds (VOCs) are released through the eggshell and can be detected and identified. The main objective of this research is to identify and characterize the VOCs emitted from infertile and fertile eggs to determine their differences using Principal Component and Linear Discriminant analyses in order to explore potential biological information such as fertilization-specific and gender-specific VOC biomarkers. 15 each of infertile and fertile eggs from non-inseminated and inseminated hens respectively were collected from the University of Georgia Poultry Research Center. The eggs were placed inside 250mL glass jars where VOCs emitted from the eggs were collected for one hour using two Stir Bar Sorptive Extraction (SBSE) devices (Twister[®]) that were magnetically attached to the lid. Sampling was conducted prior to incubation as well as on incubation days 0, 3, 6, 9, 12, and 15 respectively. VOCs were desorbed from the Twisters[®] using a thermal desorption unit (TDU) coupled to a cooled injection system (CIS) which serves both as a cryo-focusing trap as well as a temperature programmable GC inlet. Desorbed VOCs were then analyzed using a two-dimensional gas chromatograph coupled to a time-of-flight mass spectrometer (GCxGC/TOFMS) in a non-targeted approach. LECO[®] ChromaTOF[®] software was used for data acquisition. Compounds were identified using the NIST 2017 mass spectral database within LECO[®] ChromaTOF TILE[®]. Additionally, class comparison and Principal Component Analysis were also conducted using the same software package. Principal Component Analysis results indicated the ability to discriminate between fertilization statuses using headspace VOCs extracted with this method as early as day 3 of incubation. Compounds of particular significance in discriminating between fertilization statuses spanned several chemical classes including aldehydes, alkanes, ketones, and alcohols. SBSE/GCxGC-TOFMS has the potential to non-destructively characterize and discriminate VOC biomarkers that differentiate fertilized and unfertilized eggs. This fundamental work can be used to develop a fast, inline, and non-destructive tool to pre-screen eggs for fertility and eventually gender identification before being passed on to incubation.

Key Words: Egg; Fertility; Early; Non-Destructive; SBSE/GCxGC-TOFMS

474P Evaluating broiler embryo colonization and hatchability following in ovo inoculation of probiotic *Escherichia coli* Nissle 1917. Cassidy Morris^{1*}, Jiddu Joseph¹, Claudia Castaneda², Anuraj Sukumaran¹, Aaron Kiess³, Reshma Ramachandran¹, ¹Mississippi State University, Moulton, Alabama, United States, ²Engrain LLC, Manhattan, Kansas, United States, ³North Carolina State University, Raleigh, North Carolina, United States.

Probiotics, direct-fed microbials, are popular alternative to antibiotics capable of improving broiler performance and reduce pathogens. *Escherichia coli* Nissle 1917 (EcN) is a commercially available human probiotic that is reported to be beneficial in a variety of species including poultry following in-feed supplementation. In ovo administration is a promising method of probiotic delivery as it allows for early colonization of beneficial bacteria in the chick. However, there is no information on EcN's efficacy when administered in ovo. Hence, the objective of this study was to evaluate the broiler embryo colonization and hatchability following in ovo inoculation of bioluminescent EcN. Four hundred and twenty eggs from a 38-week Ross708 x YPM breeder flock were divided into 6 treatments (n=70), individually weighed, and incubated under standard incubation conditions. On 10 day of incubation (doi), eggs were candled, and infertile eggs were removed. On 18 doi, all eggs were individually weighed to determine percentage egg weight loss, and manually injected into the amniotic sac with their respective treatments: 1) Non-injected, 2) Dry punch, 3) 0.1mL PBS, 4) 10⁴cfu/0.1mL EcN, 5) 10⁵cfu/0.1mL EcN, and 6) 10⁶cfu/0.1mL EcN. On 21 doi, hatchability and chick body weight were determined. Hatch residue analysis of eggs that did not hatch was conducted to determine the cause of hatch failure. Intestinal and lung samples were collected from 5 chicks/treatment for microbial quantification as well as all tissues were visualized using an in vivo imaging system (IVIS) to confirm the presence of bioluminescent EcN. Data were analysed as a randomized complete block design with level of incubator as block and egg as experimental unit by GLM procedure of SAS 9.4 with level of significance set at 0.05. Results indicated that injecting EcN irrespective of the concentration had a negative impact on hatchability as opposed to the different control treatments (P<0.0001). For the controls, the hatchability was >90% whereas EcN even at the lowest concentration of 10⁴ cfu resulted in ~0% hatchability. Late embryonic mortality was significantly higher in the EcN injected treatments as opposed to the controls (P<0.05). Further, EcN was found to colonize the intestine and lungs with counts reaching up to 8.9 logs and 7.7 logs, respectively even for the lowest concentration of EcN (10⁴ cfu) (P<0.05). In conclusion, the commercially available human probiotic EcN is found to be pathogenic to the broiler embryos and thus, is not suitable to be developed as a probiotic for in ovo use in the poultry industry. This emphasizes the necessity for further research to explore which probiotics are good candidates for in ovo administration.

Key Words: broiler; probiotic; in ovo inoculation; *Escherichia coli* Nissle 1917; bioluminescent

475P Relationship between water-holding capacity and water property parameters in woody breast meat. Hong Zhuang^{1*}, Jian Zhang², Brian Bowker¹, ¹USDA-ARS, Athens, Georgia, United States, ²Institute of Animal Husbandry and Veterinary Medicine, Beijing, China.

Woody breast (WB) directly affects water-holding capacity (WHC) as well as texture properties in broiler fillets (pectoralis major). The aim of the present study was to investigate the relationship between WHC indicators, drip loss and marinade uptake, and the water properties to provide better understanding of how the WB condition affects WHC in broiler fillets. Skinless boneless broiler fillets were collected from a commercial plant and classified into the normal and severe WB groups. Drip loss was determined with weight loss after storage at refrigerated temperature for 3 days and marinade uptake with weight gain after fillets were immersed in a NaCl-sodium tripolyphosphate solution. Water properties, including intra- and extra-myofibrillar water abundance (represented with A_{21} and A_{22} , respectively), were determined with a time-domain nuclear magnetic resonance device. The WB condition resulted in increased drip loss (9.8% versus 6.2%) and A_{22} (2.8 versus 2.0 in drip loss trials and 4.2 versus 3.9 in marination trials) and reduced marinade uptake (6.8% versus 15.4%) and A_{21} (7.1 versus 7.8 in drip loss trials and 7.6 versus 8.6 in marination trials) ($P < 0.05$). There were no significant relationships ($r < 0.34$) between drip loss and A_{21} ($P > 0.05$) but significant relationships ($r > 0.54$) between marinade uptake ($P < 0.05$) and A_{21} regardless of meat condition. There was significant and strong relationship between A_{22} and drip loss ($r = 0.68$) and significant and very strong relationship between A_{22} and marinade uptake ($r = 0.91$) in normal meat ($P < 0.05$). However, there were no relationships ($r < 0.36$) between them in the WB meat ($P > 0.05$). These results indicate that the effects of the WB condition on WHC of meat by altering water properties in the extra-myofibrillar compartment.

Key Words: myopathy; NMR; Drip loss; Marinade uptake; Broiler pectoralis major

476P Increasing egg yolk pigmentation with a combination of natural antioxidants. Paul Engler^{1*2}, Vianney Rolland¹, Trieu Nguyen¹, ¹Nor-Feed, Beaucauzé, France, ²Labcom FeedInTech, Angers, France.

Egg yolk pigmentation is a crucial parameter of egg quality in various parts of the world. Whilst high levels of carotenoids can be used to enhance this aspect, some detrimental environmental conditions might lead to a poor success of pigmentation. The aim of the present trial was to assess the impact of a patented combination of natural antioxidants on the pigmentation of egg yolk from hens raised in a hot climate. The trial was set up in Vietnam in a commercial laying hen cage barn (46wks) divided in two

groups with distinct drinking water lines. One part of the barn received a normal layer diet and drinking water (CTL, $n=2600$) and the other half of the barn was supplemented via the addition of a combination of natural antioxidants in the drinking water for 6 weeks (100g /1000L, Effer-Grape, Nor-Feed, France, EG, $n=2000$). 100 eggs were collected per group every 2 weeks until the end of the trial. The yolk color was measured blindly using a DSM color fan. Results showed no difference in pigmentation score on D0 between groups, but a significant difference appeared from D14 (+0.7pt, $p<0.001$), increased on D28 (+1.9pt, $p<0.001$) and remained on D42 (+1.6pt, $p<0.001$). The final average pigmentation scores were 8.8 ± 1.3 and 10.4 ± 1.4 respectively for CTL and EG groups. Thus, the supplementation of laying hens, raised under heat stress conditions, with this nutritional solution appears to be beneficial for the pigmentation of their yolks.

Key Words: Polyphenols; Egg yolk; Pigmentation; Carotenoid; Antioxidants

477P Effect of forming agent inclusion and probe type on shear force texture analysis of pet treats developed from broiler wing tips. Orlando Fiallos^{1*}, Jorge Romero¹, Justin Dunavant¹, J. Wesley Rogers¹, Diego Ventura Urbina¹, Haisten Smith¹, Joshua Renew¹, Jorge Banegas¹, Hilary Carrera¹, Cristopher Almendares¹, Luis Guzman¹, Said Herrera¹, Clauber Polese¹, Alexandra Caballero¹, Eric Altom², Jessica Starkey¹, Charles Starkey², ¹Auburn University, Auburn, Alabama, United States, ²Balchem Corp., New Hampton, New York, United States.

The broiler chicken processing industry produces many low value co-products such as wing tips (WT) that may be utilized by the pet food industry. Hydrocolloids, such as ALGIN which contains sodium alginate (SA) and encapsulated calcium lactate (ECL), are often used to restructure ground meat products. Texture of pet treats impacts consumer acceptability. One common analysis performed to assess the textural characteristics of pet treats is the shear force (SF) test. However, there are multiple probes that can be utilized to perform the SF test. The objective of this study was to evaluate the impact of different texture probes on values generated when performing the SF test on pet treats with and without inclusion of a forming agent (ALGIN = 2% SA and 1.70% ECL). Previously frozen WT were ground through a 4.8-mm grinder plate and mixed. Mixtures with and without ALGIN were then extruded into 63.5-mm-diameter casings, wrapped in plastic wrap, stored overnight at 4 °C, and then frozen at -20 °C for 3 h prior to slicing into 5-mm-thick discs ($n = 360$). After slicing, half the samples ($n = 90$ per ALGIN treatment) were dehydrated at 66 °C until water activity reached 0.80. Prior to dehydration, 30 samples were cut into $5 \times 25 \times 63.5$ -mm pieces to be analyzed with the Warner Bratzler V blade (WBVB) probe. All samples were held at 4 °C < 48 h prior to analysis. The shear force test was performed using a texture analyzer TA-HDplusC with a 50-

kg load cell with 4 different texture probes: TA-42, TA-43R, KRAMER, and WBVB. The TA-42, TA-43R, KRAMER probes were used to analyze raw samples ($n = 30$ per ALGIN per probe). Dehydrated samples ($n = 30$ per ALGIN per probe) were analyzed using the TA-42, TA-43R, and WBVB probes. The SF test measures total force over distance to shear (toughness) and peak force to shear (firmness). Data were analyzed as a 2-way ANOVA using SAS PROC GLIMMIX with mean separation at $P \leq 0.05$. No ALGIN \times probe type interaction was observed for firmness ($P = 0.2800$) or toughness ($P = 0.8212$) of raw treats. However, raw treats tested with the KRAMER probe generated the greatest force over distance and peak force values ($P \leq 0.0001$). In dehydrated treats, the greatest force to shear values were observed in those without ALGIN analyzed with the TA-43R probe whereas all treats evaluated with the WBVB produced the lowest values ($P \leq 0.0001$). Dehydrated treats without ALGIN tested with the WBVB probe produced the greatest force to distance values compared with all other treatments ($P = 0.0024$). Based on ability to detect the expected differences among dehydrated WT-derived treats with and without ALGIN, the TA-43R probe appears to be the most appropriate probe type for assessing toughness and firmness.

Key Words: pet food; co-products ; sodium alginate; encapsulated calcium lactate; Shear Force

478P Assessment of textural characteristics of dehydrated pet treats generated from broiler chicken wing tips using a structure forming agent with different texture analyzer probes. Alexandra Caballero^{1*}, Jorge Romero¹, Justin Dunavant¹, Said Herrera¹, J. Wesley Rogers¹, Diego Ventura Urbina¹, Haisten Smith¹, Joshua Renew¹, Jorge Banegas¹, Hilary Carrera¹, Cristopher Almendares¹, Luis Guzman¹, Orlando Fiallos¹, Clauber Polese¹, Eric Altom², Jessica Starkey¹, Charles Starkey¹, ¹Auburn University, Auburn, Alabama, United States, ²Balchem Corp, New Hampton, New York, United States.

Broiler chicken processing generates low-value co-products such as chicken wing tips (WT). Chicken WT can be converted into value-added products like pet treats using structure-forming technologies. Addition of a combination of sodium alginate (SA) and encapsulated calcium lactate (ECL) can serve as a structure forming agent (ALGIN) and provide stability to meat products. Textural attributes may be determined using the 3-point bend test (3PB), which measures the matrix capacity to maintain stability during handling. Different probes can be used to perform the 3PB, which assesses hardness (force to break), flexibility (distance to break), and stiffness (force/distance). The objective of this study was to evaluate the effect of different probes to conduct the 3PB texture analysis in dehydrated pet treats made from WT with and without inclusion of a forming agent (ALGIN; 2% SA and 1.70% ECL). Previously frozen WT were ground with a 4.8-mm grinder plate, mixed with and without ALGIN, and extruded into

63.5-mm-diameter casings. Chubs were stored overnight at 4 °C, frozen at -20 °C for 3 h, and sliced into 5-mm-thick discs. Slices were dehydrated in a convection oven at 93 °C until an internal temperature of 74 °C was reached, then at 66 °C to water activity of 0.8. A TA-HDplusC texture analyzer with 1 of 3 probes (TA-18, TA-18B, or TA-43R) was used to perform 3PB analysis on 30 slices per ALGIN inclusion per probe. Data were analyzed as a 2-way ANOVA using SAS PROC GLIMMIX with means separation at $P \leq 0.05$. An ALGIN inclusion \times 3PB probe interaction was observed for all variables measured. Dehydrated treats with ALGIN assessed with the TA-43R probe required the greatest force to break the sample, while treats without ALGIN assessed with the TA-43R and those tested with the TA-18 probe required the least force, with those assessed with the TA-18B probe being intermediate (hardness; $P < 0.0001$). Dried treats containing ALGIN assessed with the TA-18B required the greatest distance to break while all samples evaluated with the TA-43R probe and those without ALGIN tested with the TA-18 probe were the least flexible ($P = 0.0063$). Dehydrated treats with ALGIN tested with the TA-43R probe had the greatest force/distance values compared with all others (stiffness; $P < 0.0001$). As expected, use of different probes for 3PB on dehydrated pet treats with and without ALGIN differentially affected assessment of hardness and flexibility, and, therefore, stiffness. However, based on ability to detect expected textural changes among treats with different ALGIN inclusions, it appears the TA-43R probe is most appropriate for the assessment of hardness and stiffness, but not flexibility of dehydrated WT-derived pet treats.

Key Words: textural characteristics; pet treats; sodium alginate; encapsulated calcium lactate; chicken wing tips

479P Effect of forming agent inclusion and probe type on the 3-point bend analysis of raw pet treats generated from broiler wing tips. Diego Ventura Urbina^{1*}, Jorge Romero¹, Justin Dunavant¹, J. Wesley Rogers¹, Said Herrera¹, Haisten Smith¹, Joshua Renew¹, Jorge Banegas¹, Hilary Carrera¹, Cristopher Almendares¹, Luis Guzman¹, Clauber Polese¹, Alexandra Caballero¹, Eric Altom², Jessica Starkey¹, Charles Starkey¹, ¹Auburn University, Auburn, Alabama, United States, ²Balchem Corp., New Hampton, New York, United States.

Wing tips (WT) are a poultry processing co-product with reduced commercial value. WT can be used as a primary ingredient in the preparation of pet treats thus adding economic value for the poultry industry. Pet treats utilizing novel meat products need to be evaluated for different characteristics prior to moving to the development phase. Texture is one characteristic that should be evaluated in new products to determine quality. The objective of this study was to evaluate the suitability of different probes to conduct the 3-point bend texture analysis (3PB) in raw pet treats made from WT with and without inclusion of a forming

agent (ALGIN; 2% sodium alginate (SA) and 1.70% encapsulated calcium lactate (ECL)). Previously frozen WT were ground through a 4.8-mm grinder plate and mixed. After mixing, each treatment was extruded into 63.5-mm-diameter casings stored overnight at 4 °C to allow proper gelatinization, and frozen (-20 °C) for 3 h to facilitate slicing into 5-mm-thick discs for analysis. After slicing, treats were held at 4 °C until all samples could be analyzed (< 48 h). A TA-HDplusC texture analyzer was used to conduct 3PB on 30 samples from different chubs from each treatment per probe type per analysis. Square blade (SB), TA-42, and TA-43R probes were used for 3PB test to assess: hardness (force required to break the sample), flexibility (distance required to break), and stiffness (force/distance). Data were analyzed as a 2-way ANOVA using the GLIMMIX procedure of SAS and least square means were separated with the PDIF option at $P \leq 0.05$. For hardness, no ALGIN inclusion \times probe type interaction was observed ($P = 0.9188$) and the force required to break the samples using the TA-42 probe was similar to the SB ($P = 0.1744$) and greater than the TA-43R probe ($P = 0.0346$). As expected, raw treats with ALGIN were harder, more flexible, and stiffer than those without ALGIN ($P = 0.0015$). Raw treats with ALGIN evaluated using a TA-42 probe required the greatest distance required to break compared with all others ($P = 0.0002$). For stiffness, treats with ALGIN evaluated using the TA-43R probe had the lowest force/distance values, while all the treatments without ALGIN were the stiffest ($P = 0.0101$). Differences in hardness and stiffness were detectable with all 3 probe types evaluated ($P < 0.0001$). However, only the TA-42 probe allowed for detection of differences in flexibility among raw treats with and without ALGIN ($P < 0.0001$). Therefore, we conclude that the TA-42 probe is the most appropriate for conducting 3PB analysis on bone-in, WT-derived raw pet treats and illustrates the importance of proper probe selection for products with different components.

Key Words: pet food; co-products ; Encapsulated calcium lactate; meat processing; texture characteristics

480P Predicting carcass component yields for purebred turkey (*Meleagris gallopavo*) lines using a 2D imaging system. Heather Hiscock^{1*}, Emily Leishman¹, Ryley Vanderhout^{1,2} Christine Baes¹, Shai Barbut¹, ¹University of Guelph, Guelph, Ontario, Canada, ²Hybrid Turkeys, Kitchener, Ontario, Canada.

Component yield measurements are known to have moderate to high heritabilities. Therefore, it is important to predict any valuable phenotypes that are related with meat yield improvement. However, manual data collection has become problematic since labor costs can be substantially high, especially when handling a high number of samples that are needed for genetic selection. In recent years, there has been a shift towards computer vision systems to assess the size, weight, volume, and grade of poultry using non-invasive techniques during real-time processing. The aim of

this study was to develop predictive models for turkey carcass components using a 2D imaging system. Using male turkeys from four purebred pedigree lines (A-D), approximately 11,000 images were taken over two years of shackled carcasses during real time commercial processing. Lines A and B were dam lines selected for reproductive traits, while Line C was a dam line selected for both growth and production traits. Line D was a sire line predominately selected for growth. All birds were slaughtered between 20 and 24 weeks of age (average bodyweight = 19.10 kg). Whole birds were weighed prior to imaging, while each of the economically important carcass components (breast, thighs, drums) were collected and weighed individually after images had been captured. The captured images were analyzed through advanced image analysis program to determine the surface area of each carcass (i.e., in pixels). Using linear regression, the equation $\log y = -0.556 + 0.820(\log x_1) + 0.067(\log x_2) - 0.073(\log x_3) - 0.091(\log x_4)$. Where y = breast weight; x_1 = live weight; x_2 = component area; x_3 = component coordinates (length); x_4 = component coordinates (width) was derived, predicting breast weight with a correlation coefficient 0.90 between the observed and the predicted values. Adding the date of slaughter to the model improved the correlation coefficient ($r = 0.93$). Similarly, adding slaughter date as a seasonal factor had no effect on the model ($r = 0.91$). Equations were developed for both drums and thighs, however collected image data had no significant impact on predicting weights. These results represent an important first step in developing prediction models for valuable turkey carcass components using practical imaging systems. These models will also allow the industry to better collect phenotypes in a cost-effective manner while adjusting strategies to meet consumer demands.

Key Words: Pedigree; Image Analysis; Yields

481P Effect of a proprietary botanicals blend feed additive on the meat quality of commercial broiler chickens. Nathan Griffith^{1*}, Hayley Sutherland¹, Melinda Dennis¹, Stacy zuelly¹, Marisa Erasmus¹, ¹Purdue University, West Lafayette, Indiana, United States.

Broiler chickens undergo various stressors during production, such as heat stress, which can affect meat quality. This preliminary study analyzed if a proprietary botanical blend feed additive (Probiotech International, Inc.) influenced meat quality of broiler chickens when broilers were exposed to an acute heat stress event. Broilers (Ross 708; $n = 304$) were randomly assigned to one of two treatment groups (5 pens/treatment): PhytoGenics supplementation (PLD) or control fed a standard diet (CLD). Broilers were housed at a stocking density of 27 kg/m². The phytoGenic feed additive, consisting of a blend of botanicals and essential oils, was added to a standard broiler diet at an inclusion rate of 250g/tonne of feed and fed from 15 d until birds were processed at 42 d. Between 30 and 32 d, birds were subjected to an acute heat stress

event where the peak temperature reached 34 °C. Live weight of broilers was recorded prior to processing at 39 d (CLD: 1.89 ± 0.059 kg, PLD: 1.93 ± 0.059 kg). At 40-42d, birds were harvested using electrical stunning and air chilling, and hot carcass weight (HCW) was recorded. At 4h post-mortem, carcasses were fabricated to determine the percentage of wing, thigh, drum, and breast weight. Breast drip loss, pH, and color (L^* , a^* , b^*) were also examined. Data were analyzed using PROC MIXED (SAS 9.4). HCW was higher for PLD (1.61 ± 0.032 kg) than CLD (1.51 ± 0.032 kg; $P=0.022$) birds. No differences were found among

treatment groups for pH, color, drip loss or percentages of wing, drum, breast, and thigh weight. These preliminary results indicate that Phytogenics supplementation affected HCW of broiler chickens such that Phytogenics - supplemented broilers had heavier carcass weights than the control birds, with no impact on meat quality measurements.

Key Words: broiler; meat quality; stocking density; performance; botanical blend

WPSA Lecture: Practical genetic inspired tools to improve the skeletal quality of laying hens; Nutrition, management and selection

482S Practical genetic inspired tools to improve the skeletal quality of laying hens; nutrition, management and selection. Ian Dunn^{1*} *¹The Roslin Institute, University of Edinburgh, Midlothian, Scotland, United Kingdom.*

Skeletal damage is a challenge for laying hens, as the physiological adaptations for egg laying make them susceptible to osteoporosis. Estimates of keel bone damage range upwards from 20% and there are ~ 390 million laying hens in the USA alone. There is a need to address the problem on a broad front, genetics, nutrition, housing and management. We have taken a genetic approach over many years and identified a substantial genetic component (~ 40% of the trait variance) but we had failed to provide solutions. Now, we have genetic results which led to nutritional solutions, a hypothesis to aid management and practical direct genetic solutions to aid selection. A genetic selection experiment demonstrated it was possible to improve bone quality. Analysis of quantitative trait loci from these lines revealed a genetic loci with a large effect. Fine mapping hens that expressed alternative alleles at the locus. A transcriptomic analysis of tibia of hens clearly indicated that expression of the cystathionine beta synthase enzyme located at the quantitative trait loci was the cause. The substrate is homocysteine, a component of the one carbon pathway. This allowed us to hypothesise that lowering homocysteine by feeding a methyl donor would improve bone quality by reducing homocysteine. This was

tested and tibia density was improved by 10%. Studying bone quality in laying hens made us realise that the dogma that the number of eggs a hen lays is related to bone quality was not true. This is important it means we can concentrate on the factors that are important. One of these was onset of lay/puberty which has a large effect on bone quality. Puberty is a factor which can be manipulated by management as well as being determined genetically. These results are useful, but they are not a genetic solution. We need to kill hens to quantify bone quality. To solve this we developed a high throughput radiographic method to quantify bone density in living hens. Only 12% variance was attributable to the method in a repeatability analysis and detected a highly significant effect on layer hen bone density within 3 weeks of a diet change. Importantly the trait heritability is moderate to facilitate rapid genetic progress and it is correlated with post mortem skeletal quality. In conclusion, the power of genetics has led to a number of approaches to improve skeletal quality including the keel bone. Implementing these approaches will reduce skeletal damage in laying hens when combined with improvements to the built environment and nutritional optimisation. The work was funded by; ERANET, a Keel Bone Damage COST action, BBSRC, the Foundation for Food and Agricultural Research and ABVista.

Key Words: Density; Tibia; Sternum; Breaking strength

Informal Nutrition Symposium: Understanding and meeting nutrient requirements of poultry

483S Informal Nutrition Symposium: Understanding and meeting nutrient requirements of poultry. Rosalina Angel^{2*}, Mamduh Sifri³, Todd Applegate⁴, Doug Korver¹, ¹*University of Alberta, Edmonton, Alberta, Canada*, ²*University Of Maryland, College Park, Maryland, United States*, ³*Sifri Solutions LLC, Quincy, Illinois, United States*, ⁴*University of Georgia, Athens, Georgia, United States*.

Effectively and efficiently meeting nutrient requirements remains a primary concern of poultry nutritionists. Understanding the actual nutrient requirements of the bird, the ability to utilize the nutrients within the diet, and delivering those nutrients in a form that can successfully utilized to support optimum economic returns will allow the industry to more strategically meet those challenges. This symposium will give real-world industry context to nutritionists and researchers, and set the stage for new sources of poultry nutrient requirement data and effective use of that data.

Key Words: poultry; feed formulation; feed composition; nutrient requirement; feed production

484S Historical and future approaches to estimating nutrient requirements in poultry. Peter Ferket^{1*}, Kirk Klasing², ¹*North Carolina State University, Raleigh, North Carolina, United States*, ²*UC Davis, Davis, California, United States*.

The poultry industry continuously updates strains in response to market demands that are influenced by rapidly-changing social and economic drivers. As new biological and analytical tools emerge, poultry breeders are able to respond progressively more quickly to meet the market demands with a variety of strains that differ in growth performance and poultry product quality characteristics. However, updating nutritional requirements using conventional nutrient dose-response trials as done in the past is now unable to keep up with the pace of genetic and management change as experienced today because of time and cost constraints. With the recent advancements in digital and computational technologies, the use of empirical or mechanistic nutrient requirement models for the functions of maintenance, growth, and reproduction may offer a nearly immediate estimate of required nutrient intake for a variety of commercial poultry strains, especially for amino acids and energy. Simulating “normal” productive potential of a particular genetic line of poultry using the Gompertz function is the foundational requirement of estimating daily nutrient requirements, and dietary formulation specifications to meet these daily requirements depends on feed intake determined by its genetic growth potential and environmental factors. Estimating desired feed intake can be imputed from Gompertz parameters and allometric coefficients, but estimating actual feed intake as

influenced by environmental and management conditions is much more challenging unless machine learning tools can be employed to reparametrize the simulation models based on specific production systems. The advantages and limitations of historical and emerging methods of estimating nutrient requirements of poultry will be discussed.

Key Words: Modelling; Machine Learning; Nutrient Requirements; Requirement Determination

485S Minimum nutrient requirements for feed formulations – the case of P for layers. Markus Rodehutschord^{1*}, ¹*University of Hohenheim, Stuttgart, Germany*.

This presentation provides a summary of research related to studies with laying hens that involved different dietary P concentrations. Estimates of P requirements based on analysis of literature data indicate that supplementation of mineral P in layer diets can be reduced far below current standard levels. The use of exogenous phytase allows further reduction of mineral P supplements or its complete removal from the feed. However, challenges exist in the evaluation of phytase efficacy that are specific to layer diets. The high Ca concentration in layer diets and related effects on phytate degradation and exogenous phytase efficacy make Ca another factor to be considered when optimizing dietary P supply for laying hens.

Key Words: laying hen; phosphorus; phytase; diet

486S Current and future considerations on the determination of amino acid digestibility coefficients and values. Paul Tillman², William Dozier, III^{1*}, ¹*Auburn University, Auburn, Alabama, United States*, ²*Poultry Technical Nutrition Services, Buford, Georgia, United States*.

Genetic selection has improved poultry performance annually for many decades, with targeted improvements primarily focused on growth rate and feed efficiency. These continual advancements in bird performance have provided a ‘true and real’ sustainability opportunity for the poultry industry. However, to fully maximize growth, efficiency and use resources sustainably, the bird’s requirement for key nutrients must be evaluated periodically to assure needs are realized in a balanced and economically optimal (*i.e.*, “Ideal”) manner. As such, a great deal of research has focused on refining the understanding of a bird’s requirement at various stages of production. Amino acid (AA) nutrition, in particular, has evolved in parallel with genetic selection and has provided a detailed understanding of the Ideal digestible AA ratios to consider under various production goals. The Ideal AA approach is based upon the ratio of each digestible essential AA relative to digestible Lys. These digestible essential AA are provided by both

intact protein sources and commercially available AA. The opportunity of using more of these commercial AA has grown significantly over the past decade from just Met source(s), L-Lys and L-Thr - with the additional economic availability of L-Trp L-Arg, L-Val, L-Ile and Gly (in all-vegetable based feeds). These additional AA have allowed for the ability to reduce dietary protein while concurrently reducing nitrogen output into the environment and therefore further improving sustainability. While most of these commercial AA are considered to be 100% digestible, it should be fully understood that the majority of the digestible essential AA within a feed are still being provided by the key intact protein source(s) being used. The major role provided from intact protein source(s) remains factual even when its use is decreased, from meeting the next limiting AA, through the use of an additional commercial source. With this enhanced and refined knowledge of AA “requirements”, and with increased use of commercial AA, it has become more imperative than ever to have a fully comprehensive and in-depth understanding of the AA values being used within formulation matrices, particularly those provided from our intact protein ingredients. The presentation and subsequent paper will focus on potential sources and/or methods of assessing or determining total AA values and digestibility coefficients, along with each methods advantage(s) and disadvantage(s). The targeted goal for a nutritionist should ultimately be to have a dynamic system providing reliable digestible AA values, for each AA within each intact protein source being utilized, for use in a formulation matrix.

Key Words: Digestibility; Amino Acids; Reduced Protein; Coefficients; Ideal Ratios

487S Optimisation - formulating to meet the objectives of the business. Robert Gous^{1*}, ¹*University of KwaZulu-Natal, Stellenbosch, South Africa.*

Conventionally, the overwhelming emphasis in feed formulation is to ensure that the lower bounds of all nutrients are met at least cost. These minimum levels are obtained from the results of experiments designed essentially to measure the response of broilers, turkeys, laying hens or broiler breeders to a range of feeds that are, hopefully, first-limiting in the nutrient under test. The interpretation of the results of such response trials is all-important in determining the minimum levels of nutrients to be used in the formulation program. The primary objective of most businesses is to make a profit, yet when the level of a nutrient, such as an amino acid, is chosen from the results of a response trial, it is usually the amino acid concentration that maximises growth rate or feed conversion efficiency that is chosen. These two objectives do not take account of the cost of input or the value of the output, both of which are fundamental when determining profitability for the enterprise. Calculating the cost of feeding (feed intake x cost per unit weight) is complex because the level of the amino acid under test influences feed intake and hence

needs to be predicted for different strains, sexes and environmental conditions. As feed ingredient prices change, so will the cost of feeding, which will influence profitability and hence the optimum level of the amino acids in the feed. Determining the value of the output, or revenue, might appear to be straightforward, being the product of the weight of the bird and the price per unit weight, but broilers are sold in many different ways in different markets, and the optimum amino acid level will differ depending on whether the bird is sold live, dressed or further processed, as the price and the weight of the portion being sold differs in each case. Now that tools are available for predicting feed intake and the weights of different saleable parts of a broiler, it seems sensible to choose the levels of dietary amino acids to be used in a formulation program based on the objectives of the business (maximise profit) rather than to maximise growth or feed efficiency.

Key Words: feed intake; modelling; Optimisation; objective function; responses

488S Leveraging a dynamic feed composition database in diet formulation. Ryan Dilger^{2*1}, ¹*University of Illinois, Urbana, Illinois, United States,* ²*National Animal Nutrition Program, Lincoln, Nebraska, United States.*

The process of formulating diets for poultry species requires accurate knowledge of the nutrient composition of available feedstuffs. One of the most important sources of information on feed composition for poultry species has been the nutrient composition tables published in the Animal Health and Nutrition series by the National Academies of Sciences, Engineering, and Medicine (NASEM; formerly the National Research Council or NRC). The last version of the consensus study report for poultry species was published in 1994, but a currently convened committee is working on an updated report that will leverage dynamic feed composition tables developed in coordination with the National Animal Nutrition Program (NANP). The NANP is a National Research Support Project (NRSP-9) supported by the USDA that is specifically focused on curating large databases containing analytical and performance data useful in feed formulation and modeling outcomes pertinent to animal agriculture. The NANP feed composition tables are freely available through the NANP website (<http://animalnutrition.org>). The current NANP feed composition data were derived from a systematic literature review covering ~30,000 peer-reviewed articles (1995-2018) in 20 scientific journals focused on ingredients and feedstuffs pertinent to poultry nutrition. The NANP feed composition database allows a user to access verified compositional data on more than 120 feedstuffs with more than 250 analytical targets as generated from an excess of 2 million individual records, all in a mobile-friendly format. More broadly, the database can be easily filtered to display composition data for a feedstuff given a specific range of nutrient inputs, published within a given timeframe, and originating from various data sources

(peer-reviewed literature, academic laboratories, and commercial analytical laboratories). Having the ability to generate customized reports of nutrient composition by dynamically filtering the database per specific user inputs will serve to improve nutrient delivery to poultry species through precision diet formulation. Forthcoming database features will include the ability to directly integrate the NANP feed composition database into third-party applications and automate the routine importation of verified nutrient composition data from both the peer-reviewed literature and analytical laboratories.

Key Words: nutrients; feed composition; database; analytical data; NANP

489S Rapid real time analytical methods for commodity ingredients and real-time implementation into commercial diets. Keysuke Muramatsu^{1*}, Aldair Santos¹, ¹DSM, Salto, Sao Paulo, Brazil.

Operating a large feed mill requires constant process control to ensure this high feed volume meets the bird requirement as close as possible. The advances of IoT (Internet of things) have exponentially increased the data processing speed and together with the development of rapid analysis technologies made possible real-time process control. In-line NIRS captures ingredient's chemical composition with optical probes placed directly in the production lines. The collected data is used to adjust feed formulation and mitigates the effect of raw material variability on diet nutritional specification. SFT (Spatial Filtering technique) is another technology that has potential application in real

time analysis for feed mills. It recognizes particle shape and therefore can be applied in particle size and pellet quality measurements. The investment in technologies is still high, but this issue has been overcome by larger-scale feed mills and consistent process control advantages. The application of real-time process analytical technologies keeps the product quality uniform as well as may provide possible ways of optimizing the process.

Key Words: NIR; Real-time; Rapid analysis; SFT

490S Feed mill challenges – what was formulated is not what the bird eats. Charles Stark^{1*}, ¹Kansas State University, Manhattan, Kansas, United States.

The production of broiler feed is a straight forward process that involves grinding, batching, mixing, and pelleting. However, the production of research feeds requires more effort to control processing variables that can significantly change the results of the experiment. The first variable that should be considered in the process is whether diets are formulated based on book or actual values. The next variable is particle size, the particle size of the ground grain will change growth performance. The nutrient variation from batch to batch of feeds can create differences between treatments. Finally, the percentage of fines in the feeder pan will affect feed intake/wastage. The key to a successful research project begins with controlling these variables within the manufacturing process.

Key Words: Feed; Research; Pellets

Symposium: Poultry industry paradigms: Connecting the dots

491S Genetic revolution: What have we learned so far? Jesus Arango^{1*}, ¹*Hy-Line International, Dallas Center, Iowa, United States.*

Poultry production has improved remarkably in the last five decades. Behind the progress in the broiler and layer industries is an impressive increase of genetic potential of commercial birds used for meat or egg production. A pivotal moment for the evolution of the poultry industry was the diversification of genetic stock developed and selected for meat production vs. egg production. In addition, the genetic improvement of commercial poultry products relies not only on a robust evaluation program, whose core component is the intense selection process for key economic traits; but also an overall breeding program, which includes a pyramidal gene flow that uses hybridization along the multiplication stages. This multiplication process not only magnifies the genetic impact of elite animals at the top of the pyramid, but it also exploits the combining ability and complementarity of the different pure lines used in the paternal and maternal sides of the final commercial crossbred birds. Poultry production will play an increasing role in providing good quality and affordable animal protein to the growing global population thanks to its greater sustainability when compared with other livestock species. Reaching the complex societal requests will be achieved by further increasing complexity of breeding programs, more sophisticated data collection of animal welfare associated phenotypes and genomic features combined with advanced analytical methods for best accuracy of predicted breeding values. Genomic selection is delivering increase of genetic progress in poultry programs. Genomics has evolved to include medium density chips to more recently whole genome sequence, all enabled by significant cost reduction. Contrary to the original expectations, practical implementation of genomic selection requires continual collection of good quality genotypes and phenotypes, and model retraining to maximize its benefits. In the future, we may rely on advance technologies, such as vision-devices combined with artificial intelligence to process big data for real-time collection, enabling tracking of bird movements, utilization of nests, and predict health issues. The use of advanced molecular and reproductive techniques is possible but contingent on consumer acceptance and governmental regulations that will not be discussed herein. Breeding companies, for broilers and layers, are implementing complex and sophisticated genetic poultry programs, which balance productivity and fitness traits, and keep in perspective consumer demands, public perception, and the need to ensure sustainable and welfare friendly production systems, while controlling the cost of production of poultry protein for the increasing human population.

Key Words: broilers; genetic; layers

492S Nutritional developments: What is next? Phillip Smith^{1*}, ¹*Tyson Foods, Fayetteville, Arkansas, United States.*

When I was asked if I would speak about future nutritional trends effecting the poultry industry, I thought “WOW, I don’t have a crystal ball”. Anyways, I have been around a good while, and I have seen a lot of changes in how birds are grown. Certainly, the biggest factor improving live performance and carcass yield has been through genetic selection by the primary breeders. Also, improvements in housing, equipment, and overall environmental management have been huge factors in allowing broilers to reach their genetic potential. Feed formulation has adjusted as the bird’s nutrient requirements have changed and feed ingredient availability and cost are constantly changing. The presentation will cover some possible future nutritional developments in poultry nutrition.

Key Words: nutrition; poultry

493S Developing next-gen data management strategies for smart poultry farms. Lilong Chai^{1*}, ¹*University of Georgia, Athens, Georgia, United States.*

United States is the world’s largest broiler producer and the second largest egg producer (e.g., poultry and eggs had a sale value of \$40 billion in 2021). However, poultry productions are facing grand challenges of animal welfare concerns, food safety issues, and environmental impacts. The rapid growth rate of broilers is associated with welfare concerns such as leg issues and lameness. Broilers with lameness suffer behavior restrictions, physical discomforts, and impingement of fundamental freedoms. Those welfare concerns have triggered the attention of the general public and the food industry to improve broiler well-being, and well-being evaluation. Animal welfare evaluation is currently performed manually by farm workers daily or occasionally in the poultry houses, which is time consuming, labor intensive, and subject to human errors. This task calls for the design of an automated data management strategies that can monitor and analyze poultry welfare indicators automatically. Computer vision-based image data management technologies (e.g., machine learning or deep learning models for processing and analyzing poultry images) have been tested to monitor livestock and poultry welfare and production. However, it is technically challenging to do real-time data analysis and evaluation of animal welfare because it’s time consuming to process big dataset of animal images (e.g., each camera collected 15 frames of photos per second). Researchers at the University of Georgia are developing specific data management strategies and cloud computing for imaging processing and analysis to track behaviors of broilers and cage-free layers on litter floor. Floor distribution patterns such as individual birds’ moving in different zones of feeding, drinking, and resting can be quantized and used as

indicator of animal welfare or health. The data management technologies could also be applied to monitor laying hen's behaviors of perching, pecking, and floor egg laying in the cage-free houses. Those automatic data management strategies will be further innovated for developing the core component of an integrated imaging system for evaluating poultry production (e.g., body weight), health (e.g., pecking and keel bone damages), and welfare (e.g., gait score – walking ability) in commercial broiler and cage-free layer houses.

Key Words: Animal welfare; Poultry production; Data management; Machine vision

494S Poultry processing and meat quality: challenges and outlook. Casey Owens^{1*}, ¹*University of Arkansas, Fayetteville, Arkansas, United States.*

The poultry industry has had dramatic growth especially in the past two decades. The average size broiler was just over 2.2 kg twenty years ago, and now the majority of broilers produced today are over 3 kg. This increase in bird size is a result of improved performance and longer growth periods. While producing large birds can be more economical based on product output, the shift in production has led to challenges for the broiler industry. There has been an unprecedented emergence of myopathies in most commercial broilers that have negative impacts on quality. The industry needs mitigation strategies that focus on quality of meat rather than quantity alone, and this should include genetic and live production factors. There is also a need for quality-based sorting in processing plants in efforts to provide the highest quality end product to consumers, and this requires identification and detection of quality issues. Furthermore, processing plants are also faced with increased labor demands in times of labor shortages and therefore, there is a need for more innovation in the automation of tasks from live bird handling through deboning and further processing of meat. The poultry industry has a risen to meet previous challenges in the past and will be able overcome these current challenges with increased awareness of issues.

Key Words: myopathy; meat quality; processing; quality detection; innovation

495S Welfare and sustainability: An inseparable alliance? Sara Reichelt^{1*}, ¹*Aviagen North America, Huntsville, Alabama, United States.*

As the title says, animal welfare and sustainability are an inseparable alliance. The reason for this? Animal welfare actually falls under one of the commonly recognized pillars of sustainability, the social pillar. This social pillar touches on a range of topics from feeding communities and supporting and lifting up your workforce to optimizing animal welfare. Not only does animal welfare inherently fall under sustainability in this way, it so heavily impacts the other two pillars, environmental and economic. This happens through great animal welfare contributing to a healthy animal enhancing their biological efficiency. With this efficiency comes financial soundness for a company as well as decreased resources. By being good stewards of our animals, sustainability is impacted in a range of different ways that we will continue to delve into.

Key Words: welfare; sustainability

496S Consumers and their expectations: How to meet those evolving needs? Chris DuBois^{1*}, ¹*IRI, Chicago, Illinois, United States.*

Covid-19 unleashed new forces that created and accelerated new cooking and consumption trends that will impact poultry markets for the next several years. In the last year, soaring costs have resulted in price changes that add more complexity. IRI will use its unmatched consumer data sets to dissect, explain and share the outlook for poultry, the key attributes, claims and sustainability practices that will drive growth over the next five years.

Key Words: consumers; poultry market

Symposium: Enhancing their quality of life: Environmental enrichment for poultry

497S Playful poultry: what does it take and what does it mean? Ruth Newberry^{1*}, ¹*Norwegian University of Life Sciences, Viken, Norway.*

Play is a commonly recognised behaviour in mammals but, until recently, has received little attention in poultry. So what is play? Play is spontaneous, self-handicapping, non-injurious, non-stereotyped behaviour that appears energetic but relaxed. In poultry, behaviours meeting these criteria include worm running, frolicking, and sparring, which represent object, locomotory, and social play, respectively. What does it take to stimulate play? Play occurs at higher levels when the environment offers more choices, as in complex, enriched environments. In contrast, it is suppressed under conditions resulting in unpleasant affective states (e.g. feeling hurt, sick or scared). Play is also seen when conditions improve, such as when given access to more space or enrichment materials. Then what does it mean when we see poultry playing? The occurrence of play could indicate joy or relief. Thus, while reflecting positive emotions at the time of performance, it does not necessarily indicate positive welfare over the longer term. It is also unclear to what extent higher levels of play reflect higher welfare. First, we need to compare birds of the same age or developmental stage, as play levels peak in young birds. It is hypothesised that play provides “training for the unexpected”, whereby the birds learn how to recover from a loss of control. Therefore, one would expect that as they learn more about their environment and what to expect, play will decline. Second, because play takes up only a small amount of the daily time budget, it can be easy to miss if only observing the flock for a short period each day. Third, play is easily stimulated by disturbances. When walking through the house, play is occurring in the space freed up behind the person. Treatment comparisons can give different results depending on whether the birds are disturbed or undisturbed. Fourth, lumping object, locomotory and social play together under the general umbrella of play loses information about how environmental conditions are affecting play of each type. Nevertheless, at the flock level, higher play levels have been associated with better health and lower mortality, suggesting that overall play levels provide a useful general indicator of positive welfare. Methods for including play in welfare assessment protocols will be discussed.

Key Words: Behavior; Environmental enrichment; Play; Space; Poultry welfare

498S A bird's eye view: The importance of the laying hen's experience in a successful enrichment program. Richard Blatchford^{1*}, ¹*University of California, Davis, Davis, California, United States.*

Laying hens can experience both pleasant and unpleasant mental states known as affective states. Affective states are considered long lasting moods, such as contentment or anxiety, and result from an accumulation of experiences. Whether an animal is experiencing a positive or negative affective state has a direct impact on their welfare. Recent studies have attempted to better understand the relationship of affective state and changes to the environment, such as the addition of enrichment. In this context, affective states can be measured in several ways, the simplest being physiological measures of arousal and stress. However, these measures are often misinterpreted as the valence of the affective state cannot be determined. Behavioral measures such as changes in activity budgets, facial expressions (for mammalian species), or vocalizations can also be used to study affective states. These measures can be time consuming to collect, and oftentimes are not sufficiently validated for a given species. Most recently, researchers studying affective states have turned to preference and/or motivation or cognitive bias experimental designs, which also come with their own limitations. This talk will examine how researchers have studied affective states of laying hens, the limitations of the designs, and future directions for this field.

Key Words: enrichment; laying hen; affective state

499S Environmental enrichment for broiler chickens - are there beneficial effects on welfare? Ingrid de Jong^{1*}, ¹*Wageningen University and Research, Wageningen, Netherlands.*

Due to the increase in broiler housing systems with higher welfare requirements, environmental enrichments are increasingly being applied in practice. Effective environmental enrichment, thus, enrichment that meets the needs of and is actually used by the animals, can significantly contribute to animal welfare by stimulating natural behavior and improving other welfare aspects. For example, effective environmental enrichment is suggested to decrease contact dermatitis, feather dirtiness and scratches, and to improve leg strength and walking ability. These effects are suggested to be caused by decreased contact with litter, reduced fearfulness, and increased locomotor activity, respectively. In this presentation an overview is presented of the most recent literature focusing on the effect of environmental enrichment on broiler welfare other than behavior, as behavior will be discussed in a separate presentation. Differences exist between studies in the extent to which environmental enrichments may be beneficial for welfare. This may be caused by the different conditions as applied in the studies, including the type, number and combinations of enrichment present. Further, both the need for specific environmental enrichment as well

as the effects of environmental enrichment on welfare indicators may differ between fast and slow-growing broiler chickens. Finally, when comparing systems, environmental enrichment is often confounded with other factors such as stocking density, which makes it difficult to draw conclusions on the effect of environmental enrichment on broiler welfare per se. It will be discussed which factors may be important to find any positive effects of enrichment on broiler welfare other than behavior.

Key Words: broiler; enrichment; welfare; leg health

500S Stimulating and modifying broiler behavior by provision of biologically relevant environmental enrichment. Anja Riber^{1*}, ¹Aarhus University, Tjele, Denmark.

The activity level of broiler chickens decrease with age, as the time budget will be increasingly taken up by resting, i.e. sitting inactively on the floor. Even the active types of behaviors will often be performed in less physical strenuous manners. For example, feeding, which is normally performed by domestic fowl in a standing position, may be performed while sitting. This is more pronounced in individuals with walking deficiencies than in birds with no or minor walking deficiencies. Thus, part of the decrease in activity level can be explained by deterioration of walking ability. However, the barren environments that broilers typically are kept in, where the quality of the flooring material is deteriorating with age, may contribute to the drop in activity level. Studies have shown that an increase in locomotor activity can be gained by increasing the distance between water and feed or by placing barriers in the environment. Another approach is to stimulate and modify broiler behavior by providing environmental enrichment. This can be elements such as straw bales and different foraging or dustbathing substrates that are renewed regularly. A more radical change in the environment is to provide access to a covered veranda or an outdoor area where a wider range of elements, such as vegetation, insects, and sunlight, may stimulate active species-specific behavior. The extent to which the broilers exploit the environmental enrichment depends on a range of factors. As for any animal species, the enrichment provided needs to be biologically relevant to be effective. Examples are found in the literature, where types of enrichment have been provided but only used to a poor extent by the birds. Genetics play a major role, where e.g. fast growth rates and fearfulness may hamper the use of enrichments. High stocking densities appear to result in reduced locomotor activity, i.e. the space allowance influences the possibility and the effort needed to approach the enrichment. Further, the lighting schedules that broilers are exposed to are known to affect their general activity level and will therefore most likely interact with their use of enrichments. Keeping other parameters than presence of enrichment constant, the increased activity level gained when providing enrichment is likely an expression of the housing conditions better meeting the behavioral needs of the broilers. Further

benefits are improved health, e.g. fewer leg problems, and potentially decreased fear levels. Among the challenges to overcome are the money and time the farmers have to invest in purchasing and providing the enrichment. The effects on activity as well as the benefits and challenges of enrichment will be further discussed.

Key Words: Broiler; Behavior; Environmental enrichment; Activity level; Inactivity

501S What's good for the chickens and the farmers. Michael Levengood^{1*}, ¹Perdue Farms, Salisbury, Maryland, United States.

In our journey to have an effective animal care program, we based our program on 4 pillars (1) The Chickens, (2) The Farmers, (3) Openness, Transparency and Trust and (4) Continuous Improvement. As part of our continuous improvement journey, we were exploring ways to enable our chickens to exhibit their natural behaviors. We wanted to improve our chickens' environment and allow them to do what they do naturally- act like chickens. Who better to help us with that, then the farmers. They spend more time with the chickens than anyone, so why not get their expert opinion. That led us to our chicken enrichment contest and a new understanding of what a chicken and a farmer wants and needs.

Key Words: Chickens; Enrichments; Farmers; Perdue Farms

502S Enrichments in the industry: Success stories and challenges. Karen Christensen^{1*}, ¹Tyson Foods, Springdale, Arkansas, United States.

Enrichments in a commercial broiler house can be important to provide complexity and opportunities for broiler chickens to express natural behaviors. It is important that enrichments fulfill several requirements: Birds engage with the enrichment, preferably throughout the flock. The enrichment promotes a natural behavior. The enrichment does not compromise biosecurity, food safety or farmer safety. The enrichment is sustainable. It is important that enrichment testing is done in a commercial setting because it is not possible to recreate the same environment in a smaller setting like pens. Using preference testing, we have identified the enrichments that fit the criteria we have established. Our research has found that commercial broilers prefer an enrichment that allows them to hide and/or sit next to simulating safety from predators. The hiding enrichment has been overwhelming popular with the commercial broilers. The challenge now is to determine the optimal number of enrichments that provide all birds with an available enrichment and does not create frustration due to limited access. Although the enrichments may improve the mental affect of the birds, it is also important to understand any tangible physical improvements such as benefits in leg health are important to monitor and capture. Our birds are less interested in a ramp enrichment but would

access the area under the ramp reinforcing their desire to hide. They have not demonstrated much interest in roosting enrichments (boards to sit on) or pecking objects. Challenges to include enrichments in commercial houses include identifying an enrichment that will remain viable for many years, be convenient for growers to deploy and remove, and not be a hinderance to the grower that could

become a safety issue. A practical challenge to deploying enrichments in a commercial setting is the volume of the material needed. The amount of material needed can literally require miles of material!

Key Words: Broilers; Commercial; Enrichments

Symposium: Functional properties of amino acids improve health status and sustainability

503S Functional role of amino acids to improve health status in poultry. Woo Kim^{1*}, ¹*University of Georgia, Athens, Georgia, United States.*

Because the major role of amino acids is the protein synthesis in the body, the majority of amino acid research has focused on muscle accretion and egg production by supplying balanced amino acids in poultry diets. However, many other essential functions of amino acids have been recognized; especially, certain amino acids including arginine and sulphur amino acids (methionine and cysteine) support efficient growth, different tissue development, and health via modulating body metabolisms, providing precursors of essential molecules, and regulating cell differentiation. Moreover, such functionality of key amino acids becomes more substantial to maintain health status in poultry under stress conditions like heat stress, coccidiosis and other diseases. Thus, understanding of the functional roles of these amino acids will provide us novel nutrition strategies to maintain efficient growth and health in poultry.

Key Words: Amino acid; Functionality; Gut health; Bone health

504S Utilizing amino acid balance and functional properties to improve production in broiler breeder pullets and hens. Ruben Kriseldi^{1*}, ¹*Aviagen, Auburn, Alabama, United States.*

The goal of feeding broiler breeders is to ensure adequate daily nutrient intake to achieve predetermined target body weight, optimal body composition, and planned egg output. When designing diets to fit the nutrient requirements for broiler breeders in different physiological phases, such as energy and amino acids, the needs for maintenance, weight gain, and egg mass must be considered. Over time, the need for adequate daily nutrient intake for broiler breeders has evolved due to genetic progress. This has led to birds that present high appetites while also feed efficient. Though these characteristics are desirable for broilers, they bring some unintended consequences to broiler breeders. The increased appetite in pullets can shorten feed cleanup time, which increases the difficulty of keeping the flock uniform. The genetic potential for muscle deposition may cause birds to easily develop over-fleshing when diets are too dense in lysine content. Such condition is not suitable for egg production and mating activity. In contrast, providing diets poorly balanced in some amino acids can produce poor feathering, abnormal behavior, and suboptimum body composition, which in turn will reduce livability, egg production, fertility, and hatchability. Achieving optimal development in broiler breeders requires well-balanced amino acid diets. However, formulating breeder diets to satisfy adequate development and reproduction warrants a more complex and holistic approach in which ingredients

and nutrients must be judiciously integrated. Modern broiler breeders require strict control of lysine without compromising the requirement for other amino acids. A more integral list of ingredients (wheat middlings, canola meal, peanut meal, sunflower meal, soy hulls, rice hulls, oat hulls, kaolin, etc.) in addition to the common use of corn and soybean meal may be necessary not only as other sources of amino acids but also diluents. Allowing the inclusion of available feed-grade amino acids in the diet can also help accomplish the desired amino acid balance in the feed. The success of these strategies should also be accompanied by the use of multiple feeding phases as amino acid needs at each growth and production stage are different. Utilizing more feeding phases allows for more flexibility in controlling feed allocation, thus daily amino acid intake, concurrently with body weight, egg size, and flock uniformity control. While these practices are beneficial in contributing to optimum production, balancing amino acid content is only one aspect of feeding the modern broiler breeder. Energy, vitamins, and minerals are also of utmost importance in providing the best diet for these birds.

Key Words: broiler breeder; amino acid; lysine

505S Understanding amino acid functionality to advance precision nutrition and sustainability goals in poultry production. Samuel Rochell^{1*}, ¹*University of Arkansas Division of Agriculture, Fayetteville, Arkansas, United States.*

To meet evolving sustainability goals, commercial poultry producers have been charged with reducing dietary protein concentrations in attempt to mitigate excess nitrogen excretion and runoff. Given that modern broilers have robust needs for digestible amino acids in their diet, maximizing the bird's genetic potential for feed efficiency and carcass yield can be challenging when reduced protein diets are fed. Fortunately, additional feed-grade amino acids beyond those that are typically the first three limiting (methionine, lysine, and threonine) in most poultry diets have become more widely available and economical for routine use. Despite being better able to meet essential amino acid requirements using these products, bird performance and meat yield are often impaired with extensive protein reductions, despite considerable research efforts over the last decade to support this strategy. Thus, it is becoming increasingly important to understand dietary needs for amino acids that are suspected to be limiting in reduce protein diets. Moreover, several of these amino acids, including glycine and arginine, have important functional roles beyond protein synthesis that are potentially influenced by several factors including health status and diet composition. Thus, future research will likely need to evaluate outcomes beyond growth, feed efficiency,

and meat yield to better understand the functional roles and overall dietary needs for these amino acids.

Key Words: amino acid; sustainability; protein; nitrogen; broiler

506S Industry perspective: Role of amino acids in turkey nutrition – past, present and future. Randolph Mitchell^{1*}, ¹*Perdue Farms Inc., Salisbury, Maryland, United States.*

Less information is available on the requirements of essential amino acids relative to Lysine during the various stages of growth in turkeys as compared to layers and broilers. Research in turkeys has shown that lower crude protein diets have potential to deliver equivalent performance when supplemented with adequate amounts of synthetic amino acids. The inclusion of synthetic threonine in turkey diets in the last decade has allowed a modest

reduction in crude protein. The recent commercialization of synthetic Arginine, Valine and Isoleucine has made it practical for nutritionists to significantly reduce crude protein in turkey feed formulations. Due to the cost of supplementation relative to intact protein and the scarce amount of research, the incentive to adopt lower protein diets has been lacking. Changes in the regulatory environment in some states around renewable diesel have driven a surge in prices of supplement fat during 2021-2022. The increase in supplemental fat cost has hit the turkey industry disproportionately relative to other species due to high energy requirement of turkeys. This change in economic environment has provided new incentive to turkey producers to adopt higher use of synthetic amino acids to lower the dependence on supplemental fat to meet energy requirements.

Key Words: amino acids; turkeys

Symposium: The impact of dietary protein in the sustainability of poultry production

507S The impact of dietary protein in the sustainability of poultry production. Elizabeth Santin^{1*}, Frank Mitloehner², Kirk Klasing², Peter Chrystal³, ¹*Jefo Nutrition, Saint-Hyacinthe, Quebec, Canada*, ²*University of California, Davis, Davis, California, United States*, ³*Poultry Research Foundation, Hornsby, New South Wales, Australia*.

The animal agriculture industry has been described by various groups as having a greater impact on our climate than fossil fuels. However, Dr. Frank Mitloehner, UC Davis professor of animal science and air quality specialist, has pointed out that isn't what science says. He has noted that beef production, for example, contributes 3.3% of greenhouse gas emissions, while the fossil fuel dependent sectors contribute nearly 80% of all U.S. greenhouse gases. We believe that animal-based proteins, such as poultry, is the most efficient way to produce protein, given arable land and global resources. However, there are many ways that poultry producers can improve efficiency and sustainability. This symposium will focus on the impact of dietary protein (level, source) on the sustainability of poultry production. Dr. Mitloehner defines sustainability more broadly than the usual three pillars of economic, social, and environmental impacts to include animal welfare, as well as food safety and worker safety. Dietary protein in poultry feeds has a direct connection with each of those five pillars which we will explore using this expanded definition. The price of feedstuffs has increased dramatically, and depending on location, soy production can have considerable environmental impacts. Additionally, the surplus of protein in poultry feed (by formulation or due to many factors that reduce the digestibility) can increase the fermentation and inflammation in the gut. This dysbiosis can favor *Salmonella*, *E. coli*, *Clostridia* and *Campilobacter* that impact poultry health, animal welfare and food safety. In addition, the fermentation of protein in the lower intestine may increase reactive nitrogen from the excreta, affecting environmental pollutants that can impact both animal and worker welfare as well as climate warming. The objective of this symposium is to address this new view of sustainability followed by a review of dietary protein digestion and inflammation cost and by practical recommendations that could reduce the impact of dietary protein in poultry sustainability.

Key Words: Sustainability; animal wellbeing; food safety; bio-economy; environmental

508S A new view of the pillars of sustainability for poultry production. Frank Mitloehner^{1*}, ¹*University of California, Davis, Davis, California, United States*.

Our view of sustainability must broaden to better meet the needs and sentiments of consumers and society at large. Still

just as important as ever, environmental impacts of food production should be emphasized in a sustainability framework along with animal welfare, food safety, worker safety and retention, and financial viability. When one of these five pillars of sustainability falter, others often follow, underscoring the importance of considering all areas when striving toward sustainable production in animal agriculture.

Key Words: workforce; animal wellbeing; food safety; bio-economy; environmental

509S Bioavailability of protein and synthetic amino acids: Mechanisms and practical applications. Kirk Klasing^{1*}, ¹*University of California, Davis, Davis, California, United States*.

The absorption of amino acids from intact protein sources and from supplemented free amino-acids occurs at differing intestinal locations and with differing kinetics. The practical implications of these metabolic variances for the bioavailability of the total amino acids in the diet and for nitrogen excretion will be discussed. Potential ways to optimize bioavailability including protected amino acids and the timing of amino acid appearance at sites of protein synthesis will be considered.

Key Words: protein; synthetic amino acids; bioavailability; nitrogen excretion

510S Practical feed formulation for sustainable reduced-protein diets. Peter Chrystal^{1*}, ² Peter Selle¹, Sonia Liu¹, Shemil Macelline¹, Mehdi Toghyani¹, Shiva Greenhalgh¹, Jean Fontaine³, Elizabeth Santin³, ¹*Poultry Research Foundation, Hornsby, New South Wales, Australia*, ²*Complete Feeds Solutions, Sydney, New South Wales, Australia*, ³*JEFO, St Hyacinth, Quebec, Canada*.

A reduction in dietary crude protein (CP) has been achieved by the inclusion of an increasing array of supplemental non-bound amino acids (NBAA) in broiler feed. For example, in Australia and New Zealand, broiler diets are typically formulated with L-lysine, DL-methionine, L-threonine, L-isoleucine, L-valine and L-arginine. The addition of up to six NBAA in feed may lead to issues at the feedmill, due to the extra ingredients required per batch during feed manufacture and the small amounts of the last limiting NBAA required. A reduction of 39.7 g/kg in average dietary CP, without a detrimental impact on broiler growth performance was demonstrated over a series of five studies in Australia. The decline in CP was accompanied by a decrease in soyabean meal by 44.6% (from 325 to 180 g/kg) and a concomitant 28.4% increase in maize content (from 549 to 705 g/kg). An additional three NBAA were required in tandem with CP reduction, namely L-tryptophan, L-

histidine and the conditionally essential glycine. Interestingly, an equal blend of whey protein and soyabean meal as the main dietary protein, supported maximum weight gain (2089 g) and lowest FCR (1.401) in broilers from 14 to 35 days post-hatch at a total 13.1 g/kg NBAA. However, in other studies, the maximum NBAA content, supporting best growth performance, was as high as 31.1 g/kg. This suggests a maximum total amount of dietary NBAA can be added before broiler growth performance declines, but the level is not fixed and requires further elucidation. In practical, least-cost feed formulation, a “dummy nutrient” (1000 g/kg) can be added to each NBAA and a maximum bound set, so combined total dietary NBAA will not exceed this constraint. Globally, chicken meat production is set to double over the next three decades and broiler feed demand will follow. Whilst broiler growth performance will continue to improve, the demand for

soyabean meal will increase and alternative ingredients will be actively sought. Chicken meat production remains the most efficient source of terrestrial-farmed meat production and generates less carbon dioxide equivalent (CO_2 eq/kg) greenhouse gases per kilogram than alternative meat protein sources. Indeed, the median value of global warming potential (GWP) over 108 life cycle assessments for chicken was similar to fish at 3.65 kg, whilst pork was 5.77, lamb 25.58 and beef 26.61 kg CO_2 eq/kg respectively. Thus, assigning a GWP value to feed ingredients facilitates formulating broiler diets with minimum GWP, reduced soyabean meal inclusion and increased inclusion of NBAA.

Key Words: broiler; feed formulation; reduced crude protein; sustainability

Student Workshop: The scientific method: Observe, analyze, and think critically!

511S Is science being discredited by society, and how to change that. Sergio Vieira^{1*}, ¹*Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.*

Science has brought the humanity to a nowadays standard of living never seen before throughout history. The scientific method persists as the basis of all science related achievements even though, as time flies, equipment and technologies evolve toward faster responses and understandings of the problems to solve. Political interferences, however, frequently put in check the scientists, which remain the mostly credited by the society.

Key Words: Student Workshop

512S Developing scientific methods and critical thinking skills in the field of poultry science. Michael T. Kidd^{1*}, ¹*University of Arkansas, Fayetteville, Arkansas, United States.*

The aim of this lecture is to provide undergraduate and graduate students involved in poultry science tools for

improving research protocol development and execution. Critical thinking skills in terms of thesis/manuscript preparation and review will be presented.

Key Words: Student Workshop

513S Strengths and pitfalls of statistical techniques applied to modern animal science research. Anderson Alves^{1*}, ¹*University of Wisconsin, Madison, Wisconsin, United States.*

Robust statistical analyses are crucial for guiding the interpretations taken from experimental research. Nonetheless, a misuse and/or misinterpretation of statistical techniques such as hypothesis testing may lead to incorrect conclusions based on the empirical evidence. In this lecture, we will discuss the strengths and pitfalls of some commonly used statistical techniques and their impact on the replicability of modern animal science research.

Key Words: Student Workshop

Symposium: Contribution of microbiome on sustainability

514S Contribution of microbiome on sustainability. Cristiano Bortoluzzi^{1*}, ¹*DSM Nutritional Products, College Station, Texas, United States.*

The intestinal microbiome of animals is a complex ecosystem that is in constant interaction with the host as well as the external environment. A diverse range of factors, such as diet, influence the microbiome and its function, and consequently the effect that it has on the host. On the other side, the genetic and metabolic potential of the microbiome greatly impact the metabolites excreted by the animals, and the emission of gases in the poultry house. The objective of the present symposium is to provide insights outside of our current way of thinking when it comes to microbiome research, with focus on the genetic potential of the intestinal bacteria and its impact on sustainability. We understand that gaps of knowledge regarding this area of research need to be filled, and a more targeted and sustainable approach needs to be delivered to precisely modulate the microbiome and the health of poultry flocks.

Key Words: microbiome

515S Nitrogen management: Why is it important? Gregory Siragusa^{1*}, ¹*Scout Microbiology LLC, Waukesha, Wisconsin, United States.*

Uric acid is the primary source of N in the poultry litter, which is converted to urea and hydrolyzed into NH_3 in the litter environment. Ammonia is partitioned into solid, liquid and gas phases depending on the conditions of the environment. Higher litter pH increases the volatilization of ammonia from litter when pH is greater than 7, and high moisture and high temperatures are present such as the vicinity of watering lines. Therefore, the concomitant presence of high litter moisture, higher temperature, and high pH, creates conditions for an increased conversion of uric acid and urea to ammoniacal nitrogen and volatilization. Consequences of this sequence are increases in incidence of footpad lesions, and respiratory and mucosal irritation, ultimately decreasing broiler performance and economic returns and inferior animal welfare. Efficiencies of scale achieved by modern poultry science depend upon controlling the total rearing environment. This includes litter, feed/water, air/environment. Published microbiome analyses applied to the avian production environment are becoming more and more available to practitioners as well as academic workers. Similarly, the tools for generating and analyzing microbiome data is now considered to be nearly a routine exercise. The objective of this talk will be to review available literature and give insights on nitrogen management and its link with the microbiome, importance from a sustainability and health standpoint, and how modulating microbiome and ultimately functional pathways would ameliorate gas emission in poultry production.

Key Words: Nitrogen; Microbiomes; Emissions; Health; Environment

516S Can the microbiome save the planet? Microbiome action, gas production, and sustainability: All about the nitrogen. Todd Callaway^{1*}, C. Robert Dove¹, ¹*University of Georgia, Athens, Georgia, United States.*

The microbial population of the gut is an important driver of dietary energy capture in all animal species, including poultry. This gastrointestinal microbiome encodes a vast biochemical resource that can be manipulated to improve efficiency of animal production, and can improve sustainability of food animal production. Carbohydrate fermentation of structural and non-structural carbohydrates results in production of Volatile Fatty Acids (VFA) that are metabolized for energy by the animal. However, VFA production results in the production of the greenhouse gases CH_4 and CO_2 , which are inextricably linked with gastrointestinal fermentation and cannot be entirely eliminated. VFA production can be reduced as we modify the gastrointestinal fermentation, via probiotic approaches, enzymes, post-biotics, organic acids, natural botanicals, or other interventions. In poultry, microbial cell protein synthesis occurs in the caecum and, while some Microbial Crude Protein (MCP) undoubtedly is absorbed higher in the gut via reverse peristalsis, much of the MCP synthesized is lost to the host. Non-protein nitrogen is also excreted by poultry and is a N stream that flows separately from fecal N. However, this process also produces excess ammonia that represents a significant environmental pollutant that can be reduced by modification of the microbial fermentation process. Altering the fermentation patterns in the hindgut of monogastrics can be useful to minimize N losses and improve the precision of how we feed poultry. Because ruminant animals are dependent on a pre-gastric fermentation, understanding the role of the microbial population in their nutrition and methods to improve efficiency offers clear perspectives on the role of the microbial ecosystem. This can be compared with poultry, especially in regard to nitrogen retention to maximize the advantages inherent to poultry production.

Key Words: microbiome; nitrogen

517S Role of feed additives on sustainability: Interconnection between microbiome functional changes and animal welfare. Jack Geremia^{1*}, ¹*DSM Nutritional Products Inc., Lexington, Massachusetts, United States.*

Removal of in-feed Antibiotic Growth Promoters (AGPs) sent a strong signal for the development of additives capable of replacing AGPs, securing both growth-promoting and pathogen controlling effects. At the very beginning, it did not appear likely that anything would match the benefits of

AGPs to both industry and animal welfare. Years later, the industry is still strong, and a range of products, including probiotics, prebiotics, enzymes, phytogenics, organic acids, vaccines, immune-related products and others, have filled, at least partially, the role AGPs once had. However, increasing consumer demands for more sustainable production is bringing the challenge of pathogen control to another level, and the need for novel approaches to improve gut health and pathogen control was never higher. Targeting the modulation of microbial pathways makes the

development of future gut health products more likely to succeed. The functional capability of the gut microbiome community is a reproducible, stable and a feasible target. Novel modulators offer the plasticity required for more precise functional targeting. Here, we will present the benefits of one such system targeting both functional and taxonomic control of pathogenicity.

Key Words: broiler microbiome; metagenome; microbial metabolites

Symposium: Better teaching through science: Incorporating the scholarship of teaching and learning

518S A SOTL roadmap for poultry science educators. Elizabeth Karcher^{1*}, ¹*Purdue University, West Lafayette, Indiana, United States.*

The scholarship of teaching and learning (SOTL) provides a framework for educators to evaluate student learning as a scholarly inquiry and disseminate the results to a larger audience. Educators can ask a question, collect data, analyze findings, and utilize the results to make evidence-based decisions and changes in their classroom. The field of SOTL continues to grow and offer opportunities for poultry scientists to explore their classrooms much like they would their disciplinary research. This presentation will focus on the various types of scholarly teaching, explain how to conduct a SOTL project, and provide examples of ways to reflect on and disseminate outcomes.

Key Words: Undergraduate Education; Teaching Excellence; Scholarly Teaching

519S The science of learning: A review of active learning in non-active settings. Dawn Koltes^{1*}, ¹*Iowa State University, Ames, Iowa, United States.*

It is well recognized that engaging students in active learning through hands-on activities leads to increased application, and retention of material. In poultry and animal science curricula, we observe increased engagement with material in our courses with a laboratory-based component and often hear and observe students discussing material, asking questions, and expressing an increased appreciation for the material. Unfortunately, not all courses required or offered can provide this hands-on component due to a variety of reasons, including but not limited to content of the material, size of the class, layout of the instructional space, and delivery format. In these cases, we observe more passive learning as instructors often defer to delivering the material in a continuous lecture-based manner with the majority of the information given on slides prior to class. To combat passive learning in a non-active setting, implementation of active learning techniques can increase engagement with the material. These techniques range from simple and small changes to larger overall changes to the course structure. Examples of simple changes include encouraging students to take notes during class; taking time for students to note-share in class; and implementing “think-pair-share” where student are presented with a question and ask to think on it individually, share with a neighbor, then be ready to talk to the class. Examples of changes in course structure that encourage active learning through application of material include “flipped courses” where students are responsible for viewing material prior to class then being prepared for a discussion or activity based on that material. Other examples include: team-based, problem-based, project-based learning and

problem-based learning approaches where a student or team of students are provided a problem, project, or case study to identify a solution to the problem based on information presented in class; or role-play where student-instructors roles are reversed and students are responsible for course content. While there are many active learning techniques that can be used in a variety of settings, it is important when implementing them to understand the time commitment by the instructor to create/re-create the assignment, the amount of time it takes to implement in the classroom, how students will be held accountable for their learning, and does it fit the learning objectives of the course.

Key Words: cooperative learning; active learning; scholarship of teaching and learning; teaching

520S Capitalizing on Covid-19 restrictions to improve engagement between undergraduate students and the real world of animal agriculture. Frank Robinson^{1*}, Martin Zuidhof¹, Leanna Grenwich¹, ¹*University of Alberta, Edmonton, Alberta, Canada.*

Did your laboratory sessions have to change during the Covid-19 pandemic? If so, was the change an improvement or a step backwards? Typically, the introductory animal agriculture class at the University of Alberta has bussed groups of 25 to 35 students to commercial farms three times during the term for a 60-90 minute visit. Covid-19 restrictions changed all that. The teaching team asked “what can we do better in an on-line learning environment than we have typically done in person with 140 students”? Instead, we had 48 farmers appearing online to provide virtual farm tours. Students were assigned to one of 36 groups of 3-4 students and tasked with developing a virtual farm (feeding, breeding, marketing, technology, emergency management etc.). Students were polled 9 times during the project to gain perceptions on level of engagement with the project, feelings of being part of an effective online community, ability to access instructor support/mentoring and overall level of satisfaction relative to other courses they were currently enrolled in. Students consistently reported very high levels of engagement and their responses did not vary throughout the 8-week project. They were very positive that an effective online learning community had been formed and that they enjoyed course. Online tours made it possible to showcase more farms representing a more diverse view of the Alberta animal and poultry industries.

Key Words: online learning; community; agriculture; engagement; experiential learning

521S The scholarship of teaching and learning in the promotion of tenure process. Todd Applegate^{1*}, ¹*University of Georgia, Athens, Georgia, United States.*

While many universities have broadened opportunities for faculty to develop their scholarship in teaching, means to recognize and evaluate this excellence in promotion and tenure dossiers has substantially lagged, and inconsistently defined across institutions. Having often been asked to be an external evaluator for promotion of candidates, there is a broad range of guidance across institutions, ranging from me having to request prior courses a faculty candidate had taught, to another with a teaching portfolio comprised of student, peer, and institutional evaluations, narratives of effective positioning of learning outcomes into the course and reflection on inclusive pedagogical approaches to reach a broad student demographic. Teaching portfolios, fortunately, are becoming more commonplace, as debate on student evaluations alone as scholarship of teaching evidence were questioned in the 1990s for their independence, bias, and subjectivity. Since 2000,

institutions began requiring multiple sources of evidence of teaching excellence, inclusive of more than one component of the aforementioned teaching portfolio. Fast forward to today, the conversation has matured beyond that of the teaching popularity contest (with students and/or peers) to include evidentiary measures of contribution of the candidate to course student learning outcomes (SLO), as well as positioning of the course(s) into curricular SLO (and desire/flexibility to alter pedagogy and/or approaches to course SLO). More recently, some institutional guidance for promotion has trended towards requiring the inclusion of evidence of fostering student success within and outside of the classroom.

Key Words: Teaching and Learning; Promotion and Tenure; Faculty

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