

Scotland's Rural College

Effect of amino acid supplementation to reduced crude protein diets on growth performance, organ weights and nutrient digestibility of broiler chickens exposed to sub-clinical enteric health challenges

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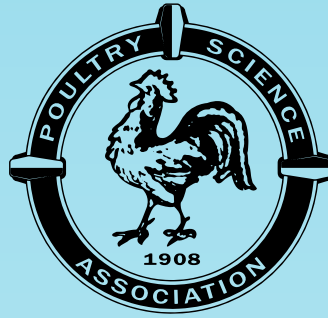
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2021 PSA Annual Meeting

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ABSTRACT BOOK

Abstracts of the Poultry Science Association 110th Annual Meeting (Virtual) July 19-22, 2021

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Animal Well-Being and Behavior

1 Withdrawn.

2 Effects of enriched floor housing and battery caging on molecular indicators of chronic stress in Bovans brown laying hens. Andrew M. Campbell*¹, Alexa Johnson³, Mike Persia², Leonie Jacobs³; ¹*Animal and Poultry Science, Virginia Tech, Blacksburg, Virginia, United States*, ²*Virginia Tech, Blacksburg, Virginia, United States*, ³*Animal and Poultry Sciences, Virginia Tech, Blacksburg, Virginia, United States*.

Plasma corticosterone (CORT) concentrations are commonly used as the gold standard for stress-related studies, however plasma concentrations show large variability making it a poor indicator of chronic stress. Immunoglobulin-A (IgA), and feather CORT have responded to known stressors in previous studies and show potential as viable biomarkers for chronic stress. However, IgA has not yet been tested in a poultry context, and few studies evaluated feather CORT as a biomarker for chronic stress in poultry. Conventional battery cages (BC) can negatively impact laying hen welfare as birds are restricted in their ability to show highly motivated behaviors, resulting in chronic stress. Enriched floor (EF) housing does allow for the expression of these behaviors. The objective of this study was to investigate plasma IgA, fecal IgA, and feather CORT responses in layer hens raised in BC and EF housing. Bovans brown hens (n=186) were randomly divided into 4 EF pens (23 birds/pen) or 28 BC (3 birds/cage). EF pens included 10 nest boxes, PVC perches, 1 hay bale, and cabbage provided 2x/week. At weeks 29 and 43 of age, plasma samples (n=12/treatment) were taken to determine plasma IgA concentrations. At weeks 21, 25, 29, 39, and 43 of age, pooled fecal samples (n=4/treatment) were collected to determine fecal IgA concentrations. Week 43 fecal samples could not be assayed. At weeks 21, 25, 29, and 43 of age, tail feathers (n=12/timepoint: 6/treatment) were cut to determine CORT concentrations. Samples were assayed using commercial ELISA kits. Plasma and feather samples were collected repeatedly from the same birds. Data were analyzed using mixed models (housing system and week as fixed factors; bird and housing ID as random factors). Data are presented as LSmeans±SEM. Plasma IgA concentrations tended to be higher in EF pens than in BC at week 29 (60.3 ng/μL vs. 44.1 ng/μL; P=0.0501) but did not differ in week 43. Hens housed in EF pens showed higher fecal IgA concentrations across all ages when compared to BC hens (423.5±45.5 μg/g vs. 295.0±44.5 μg/g; P=0.0300). Additionally, EF hens showed lower feather CORT concentrations across all ages compared to BC hens (0.042±0.005 pg/mg feathers vs. 0.062±0.006 pg/mg feathers; P=0.0085). Low environmental temperatures and

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GS Graduate Student Presenter

UG Undergraduate Student Presenter

feather loss in BC layers could have contributed to high feather CORT concentrations as those have been linked previously. These results indicate that fecal IgA and feather corticosterone show promise as sensitive and valid biomarkers for chronic stress related to laying hen housing systems. However, additional validation is needed to ensure that fecal IgA concentrations are due to chronic stress and not differences in immune status between treatments.

Key Words: IgA, corticosterone, layers, biomarker, enrichment

3 High and low stocking density effects on floor-raised pullet welfare. Meagan Abraham*, Kailynn Scoles, Marisa Erasmus, Gregory S. Fraley, Darrin M. Karcher; *Animal Science, Purdue University, Lafayette, Indiana, United States*.

The pullet phase is crucial to the lifetime success of laying hens. Management choices made during this phase may have lasting effects on the birds' physiology, behavior, growth, and lifetime production. However, the pullet phase has limited established welfare and management guidelines. This study used two different stocking densities to identify markers of welfare in pullets; hypothesizing that pullets housed at tighter stocking densities would have poorer bird-based outcomes. Using a 2x2 factorial design with Lohmann Brown-Lite or Lohmann LSL-Lite pullets at high (437 cm²/bird) or low (1059 cm²/bird) stocking density, twelve replicates of stocking density/strain combinations were housed across 48 floor pens. These pullets were followed from 0-16 weeks of age. Group size, feeder space, and water space were held constant across stocking densities. Feather coverage, keel and footpad scores, and shank length were measured monthly. Pen feed consumption and body weights of approximately 17% of all birds in this study were taken weekly. The liver was removed from approximately 20 birds per stocking density/strain combination at 1, 3, 6, 12, and 16 weeks of age. No birds were added to maintain stocking density in the study due to a confounding effect of stress from the addition of new birds. A linear mixed model one-way ANOVA was used for body weight and shank length. Strain, stocking density, and age were fixed effects and room and pen, with pen nested under room, were random effects. A linear model one-way ANOVA with strain, stocking density, and age effects or strain and stocking density effects was used to analyze uniformity and FCR respectively. There was a significant age effect (p<0.001) on uniformity, body weight, shank length, and relative weight of the liver. There were significant strain effects (p<0.05) on FCR, body weight, shank length, and relative liver weight. There was a significant strain by stocking density effect (p<0.05) on uniformity and body weight. Kruskal Wallis Rank Sum tests were used to evaluate feather coverage, footpad, and keel scores. Footpad and keel scores as well as neck, vent, and leg feather scores were perfect for the duration of the study. Age of bird had a

significant effect on back, wing, tail, and total feather coverage ($p < 0.001$). Strain had a significant effect on wing coverage ($p = 0.02$). Overall, the two stocking densities used in this study had minimal adverse effects on the pullets' health and welfare- no parameters were affected by stocking density alone. Further research is needed to identify markers of welfare in pullets.

Key Words: pullet, stocking density, welfare, stress

4 Resource-use of laying hens in a free-range setting in response to a mobile robot. Lindsey Davis^{*1}, Bulent Koc², Ahmed B. Ali¹; ¹*Animal and Veterinary Sciences Department, Clemson University, Clemson, South Carolina, United States*, ²*Department of Agriculture Science, Clemson University, Clemson, South Carolina, United States*.

Advances in robotics promote interest in applications in the egg industry to reduce human labor. However, there are concerns regarding potential bird stress stemming from bird-robot interactions. The objective of this study was to test the influence of using a mobile robot to restrict hen access to the range in a free-range setting, on resource-use, and range access of hens. Two free-range pens were used with 16 Brown Leghorn hens, 40-weeks old in each; doors to range areas were closed daily at 20:30 and opened at 06:30. A mid-sized robot (L:50×W:30×H:20 cm) was placed in one pen (ROB) to prevent hens from exiting the door to the range area during the morning (06:30 to 11:30). The robot was programmed to move forward and backward on the litter area in front of the range door continuously during the morning period. Hens in the second pen (CON) were not exposed to a robot and could be supposed to freely exit the range door after it opened in the morning. The robot was placed in the test pen during the night range restriction started at 6:30 the next day and continued for two weeks. Bird resource-use (number of birds on litter, nestboxes, and perches), and range access in both pens, were recorded daily via hourly scans during the morning period, using ceiling-mounted cameras for two weeks. Differences in resource-use and range access, across days between ROB, and CON pens, were assessed using GLMM with Tukey's Post hoc test applied to significant results in R 3.3.1 (α set at 0.05). The robot successfully prevented hens' access to the range area during morning hours and throughout the 2-week test period. In the ROB pen, no hens (0%) were able to access the range, while on average, 62.5% of the hens in the CON pen accessed the range daily ($P \leq 0.05$). On the first day of observation, more hens (67.8%) were observed perching in the ROB than CON (6.3%) pen ($P \leq 0.05$); however, by the sixth day after robot introduction, the percent of hens perching was similar in both treatments (ROB: 10.23, CON: 6.86; $P = 0.12$). Similarly, fewer hens (6.25%) were observed on litter in the ROB pen on the first day when compared to the CON pen (25%; $P \leq 0.05$). However, the number of hens on litter areas increased gradually to 53% by the third day of observation in ROB pen, compared to 18.75% in CON pen ($P \leq 0.05$). On average, 25% of hens in

the ROB pen were observed inside nestboxes daily during morning hours compared to 10% in the CON pen ($P \leq 0.05$). Thus, the mobile robot used in the current preliminary study successfully restricted hen access to range areas and improved nest use. Disturbances in perch and litter use in response to robot introduction were diminished gradually and disappeared by the sixth day of the trial.

Key Words: robot, resource-use, free-range, hens

5 Laying hens do not modulate flapping flight performance after symmetric wing feather loss. Brianna M. Leon^{*1}, Bret Tobalske², Neila Ben Sassi¹, Renee Garant¹, Donald Powers³, Alexandra Harlander¹; ¹*Animal Biosciences, University of Guelph, Guelph, Ontario, Canada*, ²*Division of Biological Sciences, University of Montana, Missoula, Montana, United States*, ³*Department of Biology, George Fox University, Newberg, Oregon, United States*.

Feathers grant most birds flight capabilities, and any loss or damage to flight feathers of the wing can impact flight performance. Laying hens experience feather damage and loss due to collisions and feather pecking, and they have naturally high wing loading and limited flight capabilities. Yet, when housed in aviaries, hens require flight feathers to navigate three-dimensional spaces to access resources. Our research aims to determine whether symmetric wing feather loss impacts flapping-flight performance in laying hens, so we modified wing areas using feather clipping. We used high-speed video to measure wing and whole-body kinematics in 18 adult laying hens 34 weeks of age to test for changes in power output and trajectory during flapping-flight descent. The hens were randomly placed into one of three treatment groups: unclipped (control, fully feathered), half-clipped (bilateral primary feathers clipped), or full-clipped (bilateral primary + secondary feathers clipped). The hens descended while flapping their wings from a 150 cm tall apparatus. We used frontal views to measure wingbeat amplitude (degrees), frequency (Hz), and angular velocity of the wrist (rad s^{-1}), and their respective asymmetries and lateral views to measure descent velocity (m s^{-1}), descent angle (degrees), and vertical and horizontal acceleration (m s^{-2}). We predicted that progressive increases in wing loading due to feather clipping would cause birds to compensate with their wing motions to accomplish similar body trajectories. A linear mixed model was used to identify treatment effects and differences between treatment groups. Wing clipping status did not significantly affect any wing kinematic measures nor vertical acceleration. Half-clipped hens demonstrated a lower descent velocity ($P = 0.0337$) and descent angle ($P = 0.0372$) than full-clipped hens. Both half- and full-clipped hens exhibited significantly lower horizontal acceleration than unclipped hens ($P = 0.0031$ and $P = 0.0018$, respectively). All hens landed with a descent velocity averaging 3.94 m s^{-1} , a landing velocity 2-3X that of adept fliers. Our results suggest that intact laying hens operate at the maximal power output supported by their anatomy and

are, therefore, at the limit of their ability to modulate their wing kinematics to control flight trajectory.

Key Words: feather loss, wing kinematics, whole-body kinematics, flapping performance, laying hen

6 Commercial field assessment of broiler welfare when enriching barns with perching, pecking and resting enrichment combinations. Jessica Walsh^{*1}, Martin J. Zuidhof², Elijah Kiarie³, Kathleen E. Long¹, Chantal LeBlanc¹; ¹*Ag Ops, Maple Leaf Foods, Vienna, Ontario, Canada*, ²*PrecisionZX, Edmonton, Alberta, Canada*, ³*Animal Biosciences, University of Guelph, Guelph, Ontario, Canada*.

Complex environments that allow animals to perform natural behavior promotes better physical and mental wellbeing. Inactivity in broilers contributes to welfare concerns in the industry such as lameness and pododermatitis. Our objective was to evaluate potential health and behavioral benefits of providing environmental enrichments in commercial broiler barns. We conducted two field trials across eight barns, containing an average of 27,000 birds per flock, with replicated treatment flocks to evaluate different enrichment combinations on object utilization, bird behavior, leg health and pododermatitis compared to unenriched control flocks. Trial one compared different pairings of perching objects (rung ramp, slatted ramp, 4" wide perch, 2" wide perch) with pecking objects (straw bales, mineral pecking block). Trial two assessed a perching object (aluminum grid ramp) paired with resting objects (plastic tunnels) and pecking objects (hanging chain links). Objects were provided at a rate of 1 object per 750 square feet of usable barn space. Behavior observations were made weekly in a pre-determined observation area using scan sampling with an ethogram. Prior to slaughter, femur and tibia bones from six males and six females were subjected to bone mass and ash content analyses. During processing at the slaughter facility, 200 footpads were scored on a scale of 0 (non-existent) to 2 (severe) for pododermatitis severity every 20 minutes throughout each flock. A pododermatitis index was calculated as the number of birds having each score multiplied by the score. Data were statistically analyzed using a mixed model with season and farm as random variables. Treatments that included the mineral pecking block decreased pododermatitis index ($P=0.047$) compared to the control. Enriched treatment flocks had an increase in femur ($P=0.007$) and tibia ($P=0.042$) ash content compared to the control. The greatest impact was in trial two, with an increase in femur ash content as a proportion of bone ($P<0.001$) and femur weight as a proportion of bird weight ($P=0.007$) compared to the control. Straw bales paired with slatted ramps, increased in comfort behavior ($P=0.027$) and decreased inactive behavior ($P=0.020$). Enrichment improved welfare in commercial barns by decreasing pododermatitis, increasing activity, and increasing comfort behavior, which is presumed to indicate an improved mental state. Heavier and higher ash content suggested improved bone health and skeletal integrity.

Key Words: broilers, enrichments, leg health, pododermatitis, comfort behavior

7 The delivery matrix and number of chickens influence the behavior of broiler chickens during preference tests: Preliminary results. Victoria A. Philp, Geraldine Muñoz, Paloma Cordero, Sergio A. Guzmán-Pino*; *Departamento de Fomento de la Producción Animal, Universidad de Chile, Santiago, Chile.*

The gustatory preferences of broiler chickens for some sapid compounds have been reported in previous studies. However, to date there are no studies that analyzed preferences for compounds added in-water and in-feed at the same time, also considering different number of chickens in tests. The objective of the present work was to assess the effect of delivery matrix and chicken number on the behavior of broilers during choice-tests. A total of 24 one-day old male broiler chickens (Ross 308) were distributed by weight into 16 floor pens, with 8 pens containing one chicken per pen, and the other 8 pens containing two chickens per pen. The experimental period lasted 23 days in which chickens were fed a commercial balanced starter diet that satisfied their nutritional requirements. From days 7 to 23, pens were randomly assigned to a 2×2 factorial design considering two levels of delivery matrix (water/ground wheat) and two levels of chicken number (one/two chickens) performing preference tests. Monosodium glutamate, lysine, sucrose and calcium carbonate were used as representative compounds of umami, umami, sweet and calcium tastes, respectively. They were diluted in water or mixed with ground wheat for preference testing against tap-water or ground wheat as control options, respectively. Tests lasted 8 hours and chickens were video recorded for 10 minutes twice, at the beginning and at the middle of exposure to the solutions/feeds. The total time of approach, number of bouts, bout duration and number of pecks at the drinker/feeder containing the sapid compound was registered in each pen. Data was analyzed with ANOVA by using the GLM procedure of SAS. A greater approach time ($p=0.035$) and number of pecks ($p<0.001$) was observed when compounds were mixed with ground wheat instead of diluted in water. The number of chickens performing preference tests influenced the total approach time ($p<0.001$), number of bouts ($p<0.001$), bout duration ($p=0.018$) and number of pecks ($p<0.001$), values being higher when two chickens performed the tests rather than just one. The compounds used for preference testing did not affect any of the parameters evaluated ($p>0.328$). Similarly, no significant interaction effects between matrix \times compound or chicken number \times compound were observed ($p>0.058$). It is concluded that the behavior of broiler chickens during a preference test is influenced by the matrix in which tested compounds are offered, as well as by the number of chickens conducting the test. Preliminary, a ground wheat matrix and two chickens per pen seem to better adjust to this type of evaluation. However, further research is needed to elucidate whether this response might vary later in chickens' productive cycle.

Key Words: behavior, broiler chicken, chicken number, delivery matrix, preference

8 How does egg laying influence HPA-axis activity in turkey lines selected for growth or reproductive traits? Emily M. Leishman*^{GS1}, Nienke van Staaveren¹, Jeff Mohr², Ben J. Wood^{3, 1, 2}, Nikole E. Freeman⁴, Amy E. Newman⁴, Alexandra Harlander¹, Christine F. Baes^{1, 5}; ¹*Animal Biosciences, University of Guelph, Guelph, Ontario, Canada*, ²*Hybrid Turkeys, Kitchener, Ontario, Canada*, ³*University of Queensland, Gatton, Queensland, Australia*, ⁴*Integrative Biology, University of Guelph, Guelph, Ontario, Canada*, ⁵*Institute of Genetics, University of Bern, Bern, Switzerland*.

Feather corticosterone (FCORT) can be used in domestic poultry as a biomarker for longitudinal HPA-axis activity. Although FCORT varies between individuals, genetic lines, and even between feathers from the same individual, it is unknown why this variation occurs. Given the primary role of corticosterone as a metabolic hormone, investigating how FCORT is influenced by periods of high energy demand (e.g., egg laying) could elucidate potential reasons for these differences. The objectives of this study were to compare FCORT concentrations in turkey hens from two different genetic lines before and during egg laying and to evaluate if there are differences between two different feathers of individual birds. One line (line A) was a male line with selection focused on growth traits, and the other line (line B) was a female line with selection focused on reproductive traits. Secondary feathers 1 and 3 were collected from 100 hens (N=50 per line) at 30 weeks of age (pre-lay). The same hens were revisited during lay (45 weeks of age) to collect regrown feathers. The FCORT samples were evaluated using ELISA. Linear mixed models were used to assess the effect of genetic line (A vs. B), period (pre-lay vs. lay), and feather (secondary 1 vs. 3) on FCORT concentration. Intra- and inter-individual variation in FCORT were further described by calculating the standard deviation (SD, pg/mg) and coefficient of variation (CV, %). An increase in FCORT during lay was detected for line B ($P < 0.0001$) but not line A ($P = 0.3155$). During lay, an increase in FCORT was detected in both feather types, though secondary 1 and secondary 3 differed from one another in total FCORT concentration. The FCORT CV among individuals (53-56%, inter-individual) were typically greater than the differences within individuals (29%, intra-individual). We conclude that egg laying can influence FCORT measurements in turkeys; however, this is dependent on genetic line and feather type. Egg laying is energetically demanding, and line B produces more eggs than line A, which may explain the observed differences between the lines because of the stronger selection for egg production. This relatively non-invasive technique, when used appropriately, may provide important physiological information to research and industry, linking HPA-axis activity, reproductive investment, and genetics of female turkeys.

Key Words: glucocorticoid, breeding, biomarker, stress, Poult. Sci. 100 (E-Suppl 1)

welfare

9 Is turkey welfare evaluated? – Validation of footpad scoring systems to improve turkey welfare. Gabriella Furo*^{GS1}, Yuzhi Li², Sally L. Noll¹, Carol Cardona³; ¹*Animal Science, University of Minnesota, Saint Paul, Minnesota, United States*, ²*West Central Research and Outreach Center, University of Minnesota, Morris, Minnesota, United States*, ³*Department of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, Minnesota, United States*.

Even though footpads are evaluated for footpad dermatitis (FPD) during processing, visual scoring is known to underestimate severe lesions in broilers. Few studies have examined agreement among FPD scoring methods used on the same turkey, thus it is not known if turkey FPD misclassification occurs and if welfare is evaluated with this approach. Pads can be scored externally on live turkeys (live scoring, LS) and postmortem (PS) when pads are clean; or histologically (HS). One histological FPD scoring system exists for turkeys that is based on the inflammatory process although it is clear that injury and regeneration are present. The objectives tested the agreement between LS and PS; and among LS, PS and HS. Turkeys were sampled (n=163) at young (Y) and market (M) ages from 18 commercial flocks. Footpads were evaluated three times: 1) LS on farm; 2.) PS after euthanasia and placement of pads in formalin, and 3) HS. For the LS and PS, a 3-point scale was used (0-no lesions, 2-severe). For the novel HS, pads were scored for injury (0-no injury, 4-damage to subdermis), regeneration (0-no signs, 1-intact epidermis and dermis), predominant inflammation (0- no signs, 3-widespread heterophilic inflammation, necrosis, and vascular proliferation), and background inflammation (0-no signs, 1-lymphoplasmacytic aggregates and/or perivascular cuffs). Spearman correlation tested the relationships and only strong correlations reported ($r > 0.60$). Cohen's Kappa Statistic tested inter-rater repeatability between two scorers in PS, intra-rater repeatability in PS and HS. The inter-, and intra-rater tests indicated that scorers interpreted all scoring systems repeatable and accurately. The strong correlation between LS and PS ($r = 0.65$ and 0.77 for Y and M, respectively) indicated that most signs of FPD with LS were identified when compared to PS with the most discrepancy within the score 1 category. For M, 60% received the same (score 1) at LS and PS. In relationship to HS, stronger correlations were detected for M. Both LS and PS correlated with the injury score ($r = 0.65$ and 0.62 , respectively), and LS was associated with predominant inflammation scores ($r = 0.61$). Regeneration was seen in M samples with severe FPD. External scores of 0 and 1 were found to be more severe histologically based on injury score. Findings indicate the revision of the 3-point external system to better match HS is needed. In addition, components of injury, inflammation, and regeneration were identified histologically, indicating that FPD is a dynamic process.

Key Words: footpad dermatitis, scoring, turkey, validation,

welfare

10 Effect of eugenol supplementation on broiler performance, litter moisture and foot pad dermatitis. Ashveen Fakeer*^{GS 1,2}, Jos Houdijk²; ¹Montida Poultry Ltd, Plaine Magnien, Mauritius, ²SRUC, Edinburgh, United Kingdom.

Footpad dermatitis (FPD), a condition characterized by lesions on the footpads of poultry, has become a major welfare and economic concern globally. Eugenol, an essential oil from plant extract, has been used extensively in poultry due to its reported anti-microbial, anti-inflammatory and anti-oxidant benefits. The aim of this study was to assess the impact of supplementing a broiler diet with an eugenol-rich feed additive (Avidry^R) on litter moisture, FPD and broiler performance under commercial husbandry conditions. A pen trial with two treatments (trial and control), each having three replicates of 2250 day-old chicks was conducted in a completely randomized block design. Avidry^R was added only in the pre-starter and starter diets at a rate of 0.2%. Grower, finisher and post-finisher feeds were commercial standard diets. Bodyweight (BW) was taken on days 0, 7, 14, 21, 28, 35 and 42 by weighing a 3% representative sample of birds. Feed intake and mortality corrected feed conversion ratio (FCR) was assessed over the corresponding periods. The FPD score was assessed on days 10, 20, 35 and 42, whilst litter moisture was analyzed on days 35 and 42. Data from the experiment was analyzed in Genstat through ANOVA. There was no significant difference in weight gain (WG) for week 1 between the trial and control, though trial birds grew slightly faster (128 vs 118g; P=0.058). However, WG was significantly greater in trial pens than in control pens for week 2 (274 vs 255g; P=0.002) and week 3 (421 vs 394g; P=0.001), resulting in final BW at week 6 being greater for trial pens than for control pens (2491 vs 2383g; P= 0.01). Average weekly feed intake (AWFI) for week 1 was not significantly different between the treatments (168 vs 165g, P=0.310). However, AWFI increased significantly in the trial pens for week 2 (311 vs 303g; P= 0.008) and week 3 (577 vs 550g; P=0.004). From week 4 to week 6, AWFI did not differ (P>0.05). FCR for week 1 was lower for trial pens than for control pens (1.27 vs 1.32; P= 0.016), and by week 6, cumulative FCR in the trial had resulted in smaller values than in control pens (1.73 vs 1.78; P= 0.041). Litter moisture was reduced in the trial pens compared to the control pens, both at day 35 (42.0% vs 45.0 %; P= 0.019) and day 42 (46.1% vs 51.4%; P=0.01). This concurred with smaller FPD score in trial pens than in control pens at 35 days (18 vs 27; P=0.001) and at 42 days (32 vs 41; P= 0.001). The results obtained in this experiment indicate that the addition of Avidry^R in the diet gave better technical performance, dryer litter and lower FPD score.

Key Words: wet litter, foot pad dermatitis, eugenol, broiler performance

11 Evaluating differences in gut morphology and sickness behavior in fast and slow growing broiler chickens when infected with *Salmonella enterica* serovar Typhimurium. Ashlyn Snyder*^{GS 1}, Tim Johnson³, Cara Robison², Shawna L. Weimer¹; ¹University of Maryland, College Park, Maryland, United States, ²Michigan State University, East Lansing, Michigan, United States, ³Purdue University, West Lafayette, Indiana, United States.

Salmonella enterica serovar Typhimurium (ST) is often a non-harmful member of the chicken gut microbial community, making it a challenge to detect in broilers prior to slaughter. However, it is possible that different broiler breeds may vary in resistance to ST. The objective of this study was to evaluate differences in gut morphology and sickness behavior between fast- and slow-growing broiler breeds when challenged with ST. Ross 308 (FG) and Redbro (SG) broilers (N=312 birds) were raised in the same pen until d 7 when they were randomly assigned and exclusively placed in isolators (N=24 isolators, 11 birds/isolator). On d 14, half of the FG and SG birds (N=12 isolators) were orally gavaged with 1.3×10^8 cfu/mL ST and half received tryptic soy broth (CON). Ileal and jejunal segments were collected from 48 birds on d 7, 13, 17, 21, and 24 (days post challenge [DPC] -7, -1, 3, 7, and 10, respectively). Video was recorded for 1 hr the day before each sampling, starting at d12, and analyzed for postures and behaviors. Histology data was statistically analyzed with JMP ANOVA and behavior data was analyzed with SAS GLIMMIX. On DPC -7, all histological measures significantly differed between breeds. FG jejunum villus height - crypt depth ratio was 0.22 lower (P=0.03) than SG, and FG ileum villus height - crypt depth ratio (IVCR) was 0.35 lower (P<0.0001) than SG. On DPC 10, there was no significant difference between breeds, but there was an effect of challenge on IVCR. The IVCR of FG-CON birds was 1.40 greater (P=0.007) than in FG-ST birds, and the IVCR of SG-CON birds was 0.76 greater (P=0.007) than SG-ST birds. There was no effect of challenge on the proportion of birds sitting and no effect of breed or challenge on the proportion of birds standing. More (P<0.0001) birds of both breeds and treatments sat at DPC 2 (65.93%) and DPC 6 (66.32%), the first two DPC recordings, than at DPC -2 (53.86%) and DPC 9 (59.55%). Fewer (P<0.0001) FG birds stood at DPC 6 (24.09%), while fewer SG-CON birds stood at DPC 2 (33.06%) and fewer SG-ST at DPC 6 (27.36%). The proportion of SG birds sham foraging generally increased with age (P<0.0001) and was similar between CON and ST except at DPC 9, where more (P=0.01) SG-CON (4.11%) sham foraged than SG-ST (2.53%). However, a similar proportion of FG-CON and FG-ST birds sham foraged each day. The results suggest differences may exist between Ross 308 and Redbro broilers regarding gut integrity and sickness behavior in response to *S. Typhimurium* infection. This information may aid breeding decisions that improve *Salmonella* resistance in broilers and reduce the *Salmonella* threat to the broiler production industry and human food supply.

Key Words: broiler, Salmonella, breed, gut, behavior

12 The effect of enrichments and spotlights on broiler production and tibia morphology. Anna Magnaterra*^{GS 1}, Rosalina Angel², Shawna L. Weimer³; ¹*Animal and Avian Sciences, University of Maryland, Ellicott City, Maryland, United States*, ²*Animal and Avian Sciences, University of Maryland, College Park, Maryland, United States*, ³*Animal and Avian Sciences, University of Maryland, College Park, Maryland, United States*.

Lights and enrichments provide environmental complexity to broiler housing environments. Enrichments can potentially improve leg health and lighting has been shown to affect behavior and welfare. However, information on the effect of enrichments and lighting to tibia morphology is limited. We aimed to see if supplemental lighting and structural enrichments impacts welfare measures and broiler tibia morphology. Broiler chickens were housed in 16 pens (n=25 birds/pen) in 2 rooms. The experiment was a 2x2 factorial design with pens randomly assigned to one of four enrichment treatments (TRT): a spotlight, an enrichment, both a spotlight and an enrichment, and a control (no spotlight or enrichment). The spotlight was a pin beam light hung above the pen and the enrichment was a platform with a 5° angle ramp. Within each pen, 5 focal birds were identified (n=80). Feed consumption and mortality were recorded daily and used to calculate feed conversion ratio. Body weight was collected at four time points (placement of birds [d0], feed change from starter to grower [d10], feed change from grower to finisher [d37], and final collection [d53]). On d53, live and postmortem measures were collected on focal birds. Welfare was scored for hock burn, footpad dermatitis, and plumage condition. Post-mortem weight (g), volume (mL), and morphological measures of the right and left tibia (proximal angle, total length [mm], depth of the intercondylar areas, and width at 10%, 25%, 50%, 75%, and 90% of total length) were taken. Means and deltas (left-right) of both tibia were calculated for each bird. Data were analyzed in JMP Pro 14 using a two-way ANOVA to determine effect of TRT on production and tibia measures. There was no effect of TRT on production or welfare measures (P>0.05). Mean widths of the tibia increased with enrichments: proximal head width (P=0.02), and at 90% (P<0.01), 75% (P=0.01) and 25% (P=0.01) of total length. Tibia weight (P=0.04) and volume (P=0.03) increased with enrichments. Mean widths of the tibia decreased with spotlights at 90% (P<0.01) and 75% (P<0.01) of the total length. The depth of the lateral intercondylar areas of the proximal head were shallower with spotlights (P=0.03). To conclude, production and welfare measures were unaffected by the presence of enrichments or supplementary lighting, but differences in tibia morphology were seen at several locations on the bone. The combination of enrichment and spotlight did not impact the morphology of the tibia. Although the spotlights and enrichments did not impact production or welfare measures in this study, they did impact leg bone morphology and further research should aim to understand the impacts in a Poult. Sci. 100 (E-Suppl 1)

commercial setting.

Key Words: tibia, enrichment, lighting, welfare, broiler

13 Adoption of precision livestock farming technologies by broiler farmers. Heitor Rios*^{GS 1}, Paulo Waquil¹, Catarina Stefanello²; ¹*Center for Studies and Research in Agribusiness, Federal University of Rio Grande do Sul, Porto Alegre, Brazil*, ²*Animal Sciences, Federal University of Santa Maria, Santa Maria, Brazil*.

A questionnaire was applied to 204 broiler farmers of Southern Brazil to analyze factors that influence their adoption of Precision Livestock Farming technologies related to broiler welfare (PLF-AW). A total of 184 farmers satisfactory answered the questionnaire with 24 questions regarding socioeconomic and productive data as well as on the adoption and willingness to adopt PLF-AW technologies. Farmers were showed pictures of PLF-AW and asked on their utilization in a “yes” or “no” question. Opinions of farmers about PLF-AW technologies potentialities to improve four factors, namely animal welfare, farmer welfare, economic/productive indicators and data management were assessed through their level of agreement in a five-point-Likert scale to three statements for each factor. Factor score was calculated as the average score of the three statements. Willingness of farmers to adopt PLF-AW was measured by their level of agreement regarding statements about animal welfare improvements with the use of PLF-AW and its possible economic outcomes in a five-point-Likert scale. Socioeconomic and productive data of farmers as well as their opinions on technology potentialities were evaluated through a logistic regression using R programming language to analyze the influence of each variable on the likelihood of PLF-AW adoption. Willingness to adopt PLF-AW technologies were compared by Kruskal Wallis and Dunn test with p-value adjusted by Bonferroni at 5% significance. The logistic regression model was estimated with 13 variables, presenting Pseudo R square of 0.28 and Chisquare P-value of 0.01. Four variables of the model significantly influenced the adoption of PLF-AW technologies by broiler farmers: farmer experience, broiler slaughter weight (heavy or griller), having another activity besides broiler production for commercialization purposes and having high beliefs on PLF potentiality to improve animal welfare. Farmer experience and producing heavy broilers negatively influenced the likelihood of PLF-AW technologies adoption (P<0.05). Having another farm activity increased the likelihood of PLF-AW adoption by 5.3 times over those farmers who were strictly dedicated to broiler production (P<0.05). Having high beliefs on PLF-AW positively influenced the adoption of PLF-AW by 3.2 times for each extra point attributed in the Likert scale (P<0.05). Farmers indicated that they would not adopt PLF-AW technologies only if it represented a loss of income. In general, broiler farmers who produced griller chickens, had less experience with broiler production, had another farm activity for commercialization purpose and believed in welfare

improvements with PLF-AW technologies were more likely to adopt such technologies.

Key Words: information technology, sensors, smart farming, IoT, animal well-being

14 What are the birds playing with?: Effects of environmental enrichments on well-being measures in colony caged Japanese Quail (*Coturnix japonica*). Chirantana Mathkari*^{GS}, Rachel Dennis; *Department of Animal and Avian Sciences, University of Maryland, College Park, Hyattsville, Maryland, United States.*

Breeding purpose Japanese quail (*Coturnix japonica*) are often maintained in a barren environment with little stimulation. Enriching the birds' environment can improve their well-being, but the environmental enrichments (EE) like beads and ropes, which have been studied previously, fail to do so due to a lack of practicality and biosecurity. The aim of this study was to evaluate the effectiveness of different EEs on the well-being of breeding quail. 40 breeding colony cages of quail (1 male, 2 females/cage) were used. Treatments consisted of two categories of EEs: protective (protective hut, grass, plastic leaves hanging) and stimulatory (mirror, feeder toy, foraging mat). Using an incomplete Latin square design, each cage received one of the 6 EEs or a control (no EE), for a period of two weeks. Behaviors were recorded for 4 hours a day, 3 days a week and analyzed by scan sampling (1 scan/ 5 minutes). Body scores, body weight and egg weights were recorded at the beginning and the end of the two weeks. Eggs laid were recorded daily. Measures were analyzed using a one-way ANOVA in SAS 9.4. All EEs reduced walking as compared to the control ($P < 0.05$) but did not affect the overall inactivity levels. Protective EEs reduced walking significantly more than the stimulatory EEs ($P = 0.0072$). Compared to the control, all EEs decreased aggressive pecking ($P < 0.01$), with the feeder and the hut exhibiting the highest reduction ($P = 0.0022$ and $P = 0.0022$). All EEs, except hanging, reduced vigilance ($P < 0.001$). Feeder, hut and mirror were the most effective EEs in decreasing vigilance ($P < 0.0001$). The birds interacted more with the protective EEs as compared to the stimulatory ones ($P = 0.0401$), with no significant difference seen among the interactions with the three protective EEs ($P > 0.05$). The number of interactions with the stimulatory EEs differed amongst themselves, with the mat being significantly more interacted with than the mirror ($P = 0.0006$) and the feeder ($P = 0.0456$). Exposure to all EEs, except hut, reduced the injury scores ($P < 0.05$), with the hanging showing the highest impact ($P < 0.0001$). The EEs did not impact the feather and the footpad scores ($P > 0.05$). The birds' body weight, egg count and egg weight remained unaffected by the EEs ($P > 0.05$). Overall, the EEs reduced stereotypical behaviors and injuries, without impacting the production parameters. Interactions with the EEs highlight the presence of unwanted social interactions in barren cages, which can be avoided by the use of EEs. Our results suggest a great benefit of the use of appropriate EEs for improving well-

being of breeding colony quail.

Key Words: quail, environmental enrichment, welfare, behavior, housing

15 Mobility of laying hens in multi-tiered housing systems is affected by flight feather loss. Renee Garant*^{GS}¹, Bret Tobalske², Neila Ben Sassi¹, Nienke van Staaveren¹, Dan Tulpan¹, Donald Powers³, Alexandra Harlander¹; ¹*Animal Biosciences, University of Guelph, Guelph, Ontario, Canada*, ²*Biological Sciences, University of Montana, Missoula, Montana, United States*, ³*Biology, George Fox University, Newberg, Oregon, United States.*

For most birds, wing use is necessary to move through various environments, regulate body temperature, and escape predation. The flight feathers (FFs) present on each wing are needed for birds to sustain flight, so much so that the loss of one feather is enough to impair flight ability. For egg-laying hens housed in multi-tiered housing systems, mobility is essential to access distributed resources within elevated tiers. However, laying hens are commonly missing wing feathers due to feather-pecking behaviour, and overall wear and tear. It is unknown how the loss of FFs affects elevated resource access in laying hens. To investigate this, 120 adult laying hens (60 brown-and 60 white-feathered birds) were housed in 12 floor pens (1 strain per pen). Pens were furnished with two elevated platforms (70cm height), two perches, and two feeders and nest boxes (one of each on the ground or fixed to an elevated platform). Within each pen, hens were randomly assigned to one of three treatments: control (FFs intact), half clip (bilateral trimming of the primary FFs), or full clip (bilateral trimming of the primary + secondary FFs). An RFID system was used to record the total duration in minutes that each hen spent accessing each feeder and nest box for 48-hour intervals on week 0 (prior to treatment), 2, 4, and 6 post-treatment application. We predicted that hens would spend less time at the elevated feeder and nest box if they received the half clip or full clip treatment, with the full clip spending the least amount of time. A generalized linear mixed model was used to determine the effect of FF clipping on elevated resource access within each strain. Results of this study demonstrated that elevated feeder and nest box usage was different for each strain and was affected by wing clipping. For white-feathered hens, the full clip treatment affected elevated resource usage with approx. 40% less time at the elevated feeder ($p < 0.0001$) and elevated nest box ($p = 0.0004$) two weeks post-treatment. Brown-feathered hens predominantly ate from the ground feeder at week 0 and did so post-treatment. However, there was a decrease in elevated nest box usage for brown-feathered hens of both clipping treatments, with an approx. 55% (half clip, $P < 0.001$) and 60% (full clip, $P < 0.001$) decrease two-weeks post-treatment. These results highlight the importance of intact FFs in enabling laying hens to move freely, fly, and navigate a multi-tiered environment.

Key Words: elevated resource access, flight feathers, multi-tier housing, feather pecking, laying hens

16 The effect of early life environmental complexity on skeletal characteristics in two strains of laying hen pullets. Erin Ross*^{GS 1}, Isabela Vitiennes², Bettina Willie², Tina Widowski¹; ¹*Animal Biosciences, University of Guelph, Guelph, Ontario, Canada*, ²*McGill, Montreal, Quebec, Canada*.

Given the ongoing shift from conventional cage to alternative housing for layers, understanding the impact of the various commercial rearing systems on skeletal development in pullets is essential to improve their bone health and welfare. This study compared the effect of three styles of commercial rearing aviaries, differing in the opportunities for load-bearing exercise available during different stages of early life, and conventional rearing cages on skeletal characteristics in two consecutive flocks of Lohmann Brown Lite (B) and Lohmann Select Leghorn Lite (W) laying hen pullets. Aviary designs varied in the amount of three-dimensional space, perches, and raised platforms within brooding compartments where birds were housed for the first six weeks of life. At 6 WoA, the compartments were opened giving access to the open litter floor area, ramps, perches, and other vertical tiers. At 6, 11, and 16 weeks of age, 10-15 pullets of each genetic strain per aviary system and 7-8 pullets of each strain from conventional cages were sampled for dissection (N=502). Long bones of the wing and leg were analyzed for breaking strength, length, and diameter, and keels were analyzed for lateral area and proportion of cartilage to bone. Increasing access to diverse load-bearing activity during development was expected to yield enhanced skeletal traits compared to less complex and more restrictive environments. Statistical analyses were conducted with SAS 9.4 software using a generalized linear mixed model. Aviary-reared birds generally had stronger radii ($p \leq 0.0048$), humeri ($p \leq 0.0002$), and femurs ($p \leq 0.0339$) and larger keels ($p < 0.0151$) than cage-reared birds. Importantly, W birds in the moderately- complex aviary consistently presented with the proportionally longest ($p \leq 0.0009$) and widest wing and leg bones ($p \leq 0.0207$) of all housing styles at 6 WoA, despite reduced three-dimensional space during the brooding period relative to the most open-concept aviary. However, after the brooding compartments opened at 6 WoA, this effect disappeared. The implications of strain remain somewhat complex, though the differences observed here indicate that W birds tended to have proportionally longer ($p \leq 0.0487$), stronger bones ($p \leq 0.0176$) and larger ($p < 0.0001$), more ossified keels ($p < 0.0001$) than B birds. Thus, rearing aviary housing and genetic strain significantly impacted skeletal characteristics in pullets. Further investigation into the effect of the unique features of the various commercial systems may provide further insight into the optimal strategy for their implementation on-farm.

Key Words: aviary, rearing, poultry welfare, skeletal development, keel bone

17 Rearing aviary design and genetic strain affect spatial cognition in laying hen pullets. Ana K. Rentsch*

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^{GS 1}, Lee Niel³, Janice Siegford², Tina Widowski¹; ¹*Animal Bioscience, University of Guelph, Guelph, Ontario, Canada*, ²*Department of Animal Science, Michigan State University, Michigan, Michigan, United States*, ³*Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada*.

Cage-free housing systems for laying hens offer behavioural freedom whilst being challenging for hens to navigate. The structural complexity demands well-developed spatial cognition to ensure that hens find resources located on the different levels within the system. Said cognitive traits are established during the rearing phase and the focus of this study. We raised two flocks (1500 brown-feathered and white-feathered chicks each) of laying hen pullets in four different commercial rearing systems. Chicks were reared in barren pullet cages (C), as well as three different types of aviaries, with aviary style 1 (A1) being least complex, aviary 2 (A2) moderately complex, and aviary 3 (A3) being the most complex. Rearing aviaries mainly differed in their complexity during the brooding phase (first six weeks of life), where A1 offers two perches, A2 an elevated platform, and three perches and A3 a large swinging platform and six perches. From each of two flocks of pullets, 17 birds per rearing group and strain participated in a T-maze test (n=272) at 13 weeks of age. The goal boxes at the end of the T-mazes arms were painted either green or blue to offer a visual clue. Reward side and reward-side colour were assigned in a balanced way across all treatment groups. The rewarded goal box offered both a food reward and an escape option. Individuals were given a maximum of 15 trials of 90 sec each, less if they reached learning criteria (80% success within five consecutive trials). Number of trials needed to reach learning criteria was then analysed in R and R Studio version 3.5.2. using survival analysis (packages 'survival' and 'survminer') with strain and style and their interaction as fixed effects and flock as a random effect. White-feathered pullets learned overall faster than brown-feathered pullets ($\chi^2 = 7.88$, $p = 0.005$, white: Lower quartile (Q1)= 4 trials (tr), median (M)= 4 tr, upper quartile (Q3)= 5 tr, brown: Q1= 4 tr, M= 5 tr, Q3= 9 tr). Rearing environment affected the learning speed with a trend for A3 pullets learning faster compared to any other group ($\chi^2 = 3.1$, $p = 0.07$, A3: Q1= 4 tr, M= 4 tr, Q3= 5 tr, A2: Q1= 4 tr, M= 4 tr, Q3= 5 tr, A1: Q1= 4 tr, M= 4 tr, Q3= 5 tr, C: Q1= 4 tr, M= 5 tr, Q3= 8 tr). In conclusion, genetics and environmental complexity during rearing can facilitate spatial cognition resulting in pullets that learn resource locations faster. A hen with better spatial cognition would be expected to have an easier transition into an adult aviary, which could improve her welfare.

Key Words: laying hen, housing, welfare, cognition

18 Effects of early-life microbiota modulation on aggression, concentrations of brain serotonin and blood cytokines in egg-laying strain chickens. Yuechi Fu*^{GS 1}, Jiaying Hu¹, Heng-wei Cheng²; ¹*Animal Sciences, Purdue University, West Lafayette, Indiana, United States*,

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Accumulating evidence from human and rodent studies has indicated that modulations of gut microbiota affect host physiological homeostasis and behavioral expression. Similarly, modulation of gut microbiota could be a feasible strategy for reducing aggression and improving welfare in chickens. The objective of this study was to determine the effects of early-life cecal microbiota transplantation (CMT) on aggression, brain serotonin, and blood cytokines in egg-laying strain chickens. Chicken lines 63 (gentle) and 72 (aggressive) were used as donors and a commercial strain Dekalb XL was used as recipients for CMT. Cecal content was diluted with gut microbiome media at 1:10 before transplantation. A total of 84 d-old male chicks of Dekalb XL were randomly assigned to 1 of 3 treatments with 7 cages per treatment and 4 birds per cage (n=7): 0.1 mL saline (control), 0.1 mL cecal content solution of line 63 (63-CMT), and 0.1 mL cecal content solution of line 72 (72-CMT). CMT was conducted via oral gavage once daily from d 1 to 10, and then once weekly from wk 3 to 5. Paired aggression tests among the birds with similar BW between treatments were conducted at wk 5 and 16, respectively. Samples of blood, spleen, and hypothalamus were collected from post-tested birds at each time point for detecting CMT-induced changes of plasma concentrations of Tumor necrosis factor (TNF)- α , Interleukin (IL)-6, IL-10 and relative splenic mRNA expressions and brain concentrations of serotonin and tryptophan. Statistical analysis was carried out with R studio one-way ANOVA for parametric data and the Kruskal-Wallis test for non-parametric data. Significance was set at $P \leq 0.05$ and trends were defined at $0.05 < P \leq 0.1$. Compared to 72-CMT and control birds, 63-CMT birds exhibited less aggression at wk 5 ($P < 0.05$) with a trend of difference at wk 16 ($P < 0.1$). 63-CMT birds also had higher Concentrations of hypothalamic serotonin ($P < 0.05$) at wk 5, while the treatment difference was decreased at wk 16 ($P > 0.1$). In addition, 63-CMT birds tended to have higher concentrations of hypothalamic tryptophan at both wk 5 and 16 ($P < 0.1$). There were no treatment effects on measured cytokines at wk 5. At wk 16, plasma concentration of IL-6 was lower, but IL-10 was higher ($P < 0.05$) with a trend for lower TNF- α ($P < 0.1$) in 63-CMT birds among the treatments. 63-CMT birds also had a tendency of lower splenic IL-6 and TNF- α mRNA expression than 72-CMT birds ($P < 0.1$). These results indicate that early postnatal CMT has a potential to modify aggression in egg-laying strain chickens via regulation of central serotonergic activity and peripheral immune response.

Key Words: cecal microbiota transplantation, aggression, serotonin, immune response, chicken

19 High and low stocking density effects on the immune system of floor-raised pullets. Meagan

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The pullet phase lasts from 0-16 weeks of age and management choices during this phase have the potential to alter the birds' physiology, behavior, growth, and lifetime production. However, there are limited welfare and management guidelines during the pullet phase. Using stocking density as a stressor, this study hypothesized that tighter stocking densities would equate to poorer immunity and lower immune organ size. A 2x2 factorial design with Lohmann Brown-Lite or Lohmann LSL-Lite pullets at high (437 cm²/bird) or low (1059 cm²/bird) stocking density was used. There were twelve stocking density/strain replicates housed across 48 floor pens and the birds were followed from 0-16 weeks of age. Group size, feeder space, and water space were held constant across stocking densities. The pullets were vaccinated for Newcastle disease (NDV) at approximately 2, 4, 8 and 12 weeks of age. Blood samples were taken at 6, 9, 12, and 16 weeks of age to measure H:L ratios and NDV vaccine titers. Using approximately 20 birds per stocking density/strain combination, the bursa and spleen were removed at 1, 3, 6, 12, and 16 weeks of age and the 2nd thymus lobe of both right and left sides was removed at 1, 6, 12, and 16 weeks of age. These organs were weighed, and length, width, and height measurements taken. Body weights were also recorded from these euthanized birds to calculate relative organ weights. A linear mixed model one-way ANOVA using strain, stocking density, and age as fixed effects and room and pen, nested under room, as random effects, was used for analysis of H:L ratios, NDV vaccine titers, and organ dimensions and weights. Data was transformed as needed to best fit statistical models. At 16 weeks of age, 25 days post-breast vaccination with NDV, average IgG vaccination titers for the brown strains were 10,681 and 5,120 for the high and low stocking densities respectively while the whitestrain titers were 7,189 and 8,570 for the high and low stocking densities respectively. Vaccine titers had significant age, strain, strain x age ($p < 0.001$) and age x stocking density ($p = 0.03$) effects. There were significant strain effects ($p < 0.05$) on H:L ratio, spleen and bursal dimensions, and relative weights of the left thymus and bursa. Overall, stocking density alone did not affect immune parameters within this study. The two stocking densities used in this study had minimal adverse effects on the pullet's immune organ size and did not cause enough stress to alter immune function as measured by H:L ratio and NDV IgG titer.

Key Words: pullet, stocking density, welfare, immunity, stress

20 Effects of perch material on performance, perching times and welfare in laying hens. Ashlyn J. McIntyre*^{UG1}, Pratima Adhikari¹, Ishab Poudel², Tom Tabler³, Victoria R. Williams-Hodge²; ¹*Poultry, Mississippi State University, Starkville, Mississippi, United States*, ²*Poultry Science, Mississippi State University,*

Mississippi state, Mississippi, United States, ³Mississippi State University, ³Mississippi State, Mississippi, United States.

A 6-week study was conducted to determine what perch material was best for welfare of layer hens in a cage-free environment. Total 72 hens (6 hens per pen) at 47 weeks of age were used in this study. Out of total 12 identical cage-free pens, 6 pens had metal iron pipe while another 6 had wooden dowel as perch material. Both perch types were 6 ft long, placed at 2.5 ft distance from the litter and had 30 cm linear hen space while perching. Hens had an adaptation period of 6 days and the pens where the birds were placed contained a littered floor, waterline and a hanging feeder. Hen weights, amount of feed given, and paw/comb/feather scores were recorded at the start of the study. Eggs were collected daily and recorded for all pens to calculate Hen-Day Egg Production (HDEP). Feed consumption, hen day egg mass (HDEM) and feed conversion ratio were recorded weekly. For the final weeks of the trial, individual Hikvision® Night Vision cameras were placed in each pen to record perch placement at different time points during day and night to track how many birds were perching and data were collected. At the end of the trial, hen weight as well as their paw, comb and feather scores were recorded. Data was analyzed using a completely randomized design with split plot in time for all the variables measured and

values that were $P \leq 0.05$ were considered significant. HDEP significantly increased over the age of hens ($P=0.0063$) which indicated the birds' production was improving. Regardless of perch type, HDEM significantly increased as time passed ($P=0.0004$) where the overall mass of eggs laid by hens continued to increase from 46.6 to 59.2 from weeks 49 to 50, respectively. Feather score significantly decreased from weeks 47 to 51 with 0.319 and 0.577 values, respectively ($P=0.0075$). Paw and comb scores did not change and stayed relevantly the same throughout the experiment. There was a significant increase in hen weight over the experiment's duration ($P=0.0009$) from weeks 47 to 51 wk. Perching behavior was significantly affected by time ($P=0.0001$). At 01:00, mean percentage of birds perching was 95.75% indicating that overall, birds preferred to perch at night regardless the type of the perch material. Similarly, 100% of birds were on the floor at 07:00, 98% were on the floor at both 13:00 and 17:00 and only 4.25% of birds were on the floor during 01:00. No significant differences were found between hen preference to perch type. A longer time may be needed to acclimate birds to their environment so that the perch data is not influenced by the birds reacting to a new environment. This research drives us to keep looking to better the welfare of hens used in a laying environment.

Key Words: cage-free, laying hen, perch material, welfare

Extension and Instruction

21 Using an Excel workbook called “The Calibration Curve Confidence Calculator” (cccc.exe) for analyzing standard curve-type experiments. Gene M. Pesti^{*2, 1}, Lynne Billard³, Shubiao Wu², Thi Thanh Hoai Nguyen², Robert A. Swick²; ¹*Poultry Science, University of Georgia, Greensboro, Georgia, United States*, ²*School of Environmental and Rural Science, University of New England, Armidale, New South Wales, Australia*, ³*Statistics, University of Georgia, Athens, Georgia, United States*.

Bioassays are fundamental to understanding nutritional properties of feed ingredients and many physiological responses. Bioassays often involve standard curve-type trials and the objective is different from many other research trials. The objective of many bioassays is to estimate the mean and minimize its confidence interval for samples of unknown composition. Bioassays are not fundamentally different from chemical assays, except in the amount of variation typically observed between the responses of living organisms versus carefully controlled chemical reactions. The CCCC.exe workbook was designed to compare 4 common methods of estimating the variation in standard, or calibration curve-type results (Counter-intuitive (one-way ANOVA), Intuitive (normal regression, reverse prediction), Sophistic (reverse regression, normal prediction), and Abductive (Graybill’s Method)). Inputs include the points of the standard curve (x_1, y_1) and responses of the test samples (y_0). There can be up to 50 points in the standard curve and 10 replicates per test sample. Outputs include the mean (x_0), confidence interval, CV, SEM, Lower Limit of Detection and Lower Limit of Quantification for each method. The standard curve is transformed by 12 methods to determine the transformation (if any) that will be most helpful to linearize the standard curve. A power analysis spreadsheet using the Abductive method takes costs per observation, observations per replicate and replicates per unknown test sample and graphs expected SEMs against total cost of the experiment. The workbook is free to download from www.poultryhub.org.

Key Words: bioavailability, statistics, design, calibration, Excel

22 No presentation materials submitted.

23 Extended lay cycles: A new path to sustainable egg production in Canada. Daniella Batres*, Valerie Carney; *Agricultural, Life and Environmental Sciences, University of Alberta, Edmonton, Alberta, Canada*.

Extended lay cycles refer to the managerial trend of keeping laying hens in production for longer than the historical goal of 68 to 75 weeks of age. Managing flocks beyond 90 weeks of age is becoming common in North America, Latin America, and Europe, mainly as genetic companies aim to achieve 500 eggs within 100 weeks of lay without molting. However, flock cycle lengths in Canada have remained

steady at 12-month production cycles (67 to 71 weeks of age), only deviating to accommodate yearly peaks and valleys in chick, pullet, and specialty egg demands. Literature shows that flocks are regularly achieving 97% peak of lay and shell quality within acceptable limits at 80 weeks of age (i.e., Egg weight = 65.2 g, Haugh units = 71.7, Relative shell weight = 11.3%, Shell thickness = 404.2 μm) using linear mixed models. While the improved genetic potential (i.e., persistency of production and egg quality) of laying hens has made extended lay cycles possible, these birds require exceptional management to achieve this potential. For producers, outweighing this challenge are the economic, environmental, and social benefits of longer flock cycles, which contribute to sustainable egg production. Although the benefits are clear, the path to longer flock cycles can be complicated in Canada, which has a unique industry infrastructure (i.e., supply management). The objective of the present study was to compare the Canadian egg-laying industry to others successfully pursuing extended flock cycles and provide an in-depth understanding of what barriers exist in Canada. Research methods involved interviews ($n=20$) with industry service providers and producers across North America, Latin America, Europe, and Australia. Curation of information identified seven factors that affect the successful pursuit of longer flock cycles. These factors include the pullet rearing market (i.e., competition, availability, and flexibility), the processing industry (i.e., processor availability and egg market), and supply management (i.e., competition and innovation). Related to these factors are common challenges for producers worldwide (i.e., animal health, welfare, and shell quality) and challenges that specifically affect Canadian producers (i.e., geography, market size, and supply management). Three interviews served as informal case studies that illustrate the successful adoption of extended flock cycles and insight into producer motivations and challenges. Additionally, while many suggest that Canada’s national supply management system is the main barrier to longer flock cycles, the results of this project indicate that other factors such as infrastructure, geography, and culture play a role and may apply to other countries.

Key Words: laying hens, longer cycles, persistency, sustainability

24 Assessment of retention trends in the College of Agriculture and Life Sciences to determine possible internal recruitment efforts for Poultry Science Departments. Peyton A. Taylor*^{GS 1}, Ryan Walker², Kelley G. Wamsley¹, Jessica B. Wells¹; ¹*Poultry Science, Mississippi State University, Mississippi State, Mississippi, United States*, ²*Curriculum, Instruction, and Special Education, Mississippi State University, Mississippi State, Mississippi, United States*.

With only 6 poultry science departments remaining in the U.S. and poultry production steadily increasing, the demand

for poultry science graduates cannot be met. Therefore, recruitment in these departments is necessary to attempt to meet the industry's demand for qualified employees. Recent research from our lab has focused efforts on external recruitment practices for poultry science departments as well as methods of recruitment students prefer. However, to our knowledge, no research has assessed recruitment efforts internally within Poultry Science degree-granting institutes. With current dropout rates for university students nationwide at 40% and approximately 30% of those students being college freshman dropping out within one year; these students may be retained at their respective universities if recruited into a department that would be a "better fit" i.e., poultry science. Therefore, the objective of this study is to determine potential areas within the Colleges of Agriculture (CALs) at Poultry Science degree-granting universities where recruitment efforts could be focused on increasing retention at the university level and increasing student numbers within Poultry Science Departments. Retention rates were collected through institutions of effectiveness at two universities, and Mississippi State University's (MSU) Graduating Positioning Systems (GPS) software was utilized to assess possible trends for each department within CALs. Data was collected from Fall 2010 to Fall 2019, and retention rates were analyzed to obtain a 10-year average, along with the GPS system analyzing five-year trends (2010 - 2015). It was determined that most students within CALs at MSU tend to change majors or leave the university within their first year, resulting in an average retention rate of 59% for the college. Using data from the GPS system, it was determined that Biochemistry and Animal and Dairy Sciences have the largest population of students who change majors but still graduate from the university each year (n=50 and 17, respectively). When comparing to data collected from another Poultry Science degree-granting institute, similar trends were observed with Animal and Biological Sciences having the 2 largest populations of students changing majors within the first year. This descriptive data suggests that internal recruitment within CALs at Poultry Science degree granting institutes could be a cost-effective and easy method for Poultry Science Departments to increase student numbers as well as increase retention within CALs. For future research, data will be collected from more Poultry Science departments to provide replication and possibly confirm trends observed.

Key Words: recruitment, poultry science, retention rates

25 Assessment of current Hatch-out Program curriculum implemented in K-12 for Mississippi. Marissa Powell*^{GS 1}, Ryan Walker², Kelley G. Wamsley¹, Jessica B. Wells¹; ¹*Poultry Science, Mississippi State University, Mississippi State, Mississippi, United States*, ²*Curriculum, Instruction, and Special Education, Mississippi State University, Mississippi State, Mississippi, United States*.

Consumer education is imperative, as their
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preference/purchases heavily influence the agriculture industry. Additionally, their decisions have been linked to educational experiences in K-12 education. Therefore, a lack of agriculture education in the classroom may negatively impact the agriculture industry. Meaning implementing agricultural literacy in the classroom is imperative; however, it has been proven there is a lack of comfortability with agriculture curriculums by K-12 instructors. Mississippi State University has utilized a program (Hatch-out Program) to incorporate poultry curriculum into K-12 classrooms, and although the program has been implemented in previous years, the curriculum has not been formally evaluated for effectiveness. Therefore, the proposed objective is to determine if previous participants of the Hatch-out Program feel that it is adequate or needs improvements. An 18- question survey was delivered to previous participants (n=42) of the Hatch-out Program using Qualtrics. The participants were given 3 weeks to complete the survey at their convenience, and a reminder email was sent out weekly. Results from the survey were overwhelmingly surprising. With a 67% return rate, with 89.3% of answers indicating that teachers acknowledged a lack of confidence when it came to using and instructing their students with the Hatch-out Program, and only 3 (10.7%) teachers identifying their knowledge of the poultry industry as "Confident". In four different areas of the Hatch-out Program (materials, activities, lessons, curriculum), teachers indicated a strong confidence in feeling prepared to teach the students when given the current materials for each. However, when given the opportunity to provide open ended feedback for each part of the program, there were recommendations for improvements from some teachers with 37.5% for materials, 25% for activities, 14.3% for lessons, and 22.2 % adding comments on the curriculum. 53.3% of the teachers also identified areas of overall improvement to the program such as instructional videos serving as a teacher aid and for students to watch throughout the week with detailed explanations, more age appropriateness and more help understanding the program. With these results, a modified curriculum will be developed that will aid teachers in feeling more confident in implementing poultry curriculum into classrooms in Mississippi. This revised hatch-out program can then be evaluated for student and teacher knowledge gained, longevity of knowledge retained, and the overall effectiveness of the program.

Key Words: agriculture literacy, K-12, hatch-out program, poultry

26 An online webinar series to teach backyard flock owners about poultry viral respiratory diseases. Eliza Theis*^{GS 3}, Wayne Martin², Sally L. Noll¹, Robert Porter³, Colleen Carlson², Michaela Olson³, Abby Schuft²; ¹*Animal Science, University of Minnesota, Saint Paul, Minnesota, United States*, ²*University of Minnesota Extension, Saint Paul, Minnesota, United States*, ³*University of Minnesota, Saint Paul, Minnesota, United States*.

To educate backyard and small flock poultry owners about viral respiratory diseases, diagnostics, and prevention, the University of Minnesota (UMN) Extension Poultry Program offered a webinar series. Our objectives were to increase backyard flock owners' awareness of viral poultry diseases, how to utilize the Veterinary Diagnostic Laboratory (VDL), and biosecurity measures to prevent disease. The weekly series began mid-January 2021 and was hosted using Zoom for five weeks. Promotion of the programs was disseminated using the UMN Extension webpage calendar of events, social media and an established email distribution list. Webinar topics included: 1. anatomy and physiology of avian respiratory system 2. avian influenza and Newcastle disease 3. infectious laryngotracheitis, infectious bronchitis virus, and Mycoplasma 4. Veterinary Diagnostic Laboratory 5. biosecurity. The one-hour webinars consisted of pre-recorded lectures, followed by 30 minutes for live question and answer and group discussion. The recorded lectures were then posted on the UMN Extension Poultry YouTube channel for future viewing. As of April 30, 2021, the recordings had a total of 152 views. Attendee satisfaction was evaluated through weekly Qualtrics surveys, sent electronically within one day following each webinar. Of 109 total attendees across the five webinars, 19 survey responses were obtained for a return rate of 17%. Survey

questions asked if the information was presented clearly, if attendees felt they had a deeper understanding of the material after the presentation, and if their questions were answered clearly. Responses were reported using a 5-point Likert scale, from "strongly agree" to "strongly disagree". Answers ranged from "strongly agree" to "neither agree nor disagree", with 93% of all responses being "somewhat agree" or "strongly agree". Qualitative knowledge gain was also evaluated through the Qualtrics surveys and the attendee's activity in the chat box. Many questions were generated, in addition to general dialogue surrounding backyard and small flock poultry. Survey feedback indicated the webinar content was relevant to backyard poultry owners; 90% of respondents strongly agreed that presenters deepened their understanding of each topic. Participant survey responses and interactive chat each week demonstrated the webinars were of interest to the attendees. Future webinar suggestions included cold weather care, vaccination, parasite control, and feed additives (e.g., oregano). Overall, the format and content of these webinars was well-received and proved useful to backyard flock owners. This webinar series was funded by the United States Department of Agriculture (USDA).

Key Words: extension online, backyard poultry, respiratory disease

Genetics and Molecular Biology

27 Genome wide association study for shell color in White Leghorns using a SNP Chip and low-pass sequencing data. Anna Wolc^{*1, 2}, Janet Fulton², Zigui Wang³, Jinghui Li³, Petek Settar², Jesus Arango², Kaylee Rowland², Danny Lubritz², Hao Cheng³; ¹*Animal Science, Iowa State University, Ames, Iowa, United States*, ²*Research and Development, Hy-Line International, Dallas Center, Iowa, United States*, ³*Department of Animal Science, University of California, Davis, California, United States*.

SNP chips have become a standard tool for genomic analysis with high reproducibility and increasingly affordable pricing. However, the information is limited to a predetermined number of genomic locations depending on chip size. This limitation can be overcome by the use of low-pass sequencing where all genomic locations are interrogated but at a lower accuracy than in standard sequencing thus reducing the cost but the accuracy of calls is recovered by combining the genotype calling with imputation. Some literature suggests that using low-pass sequencing can improve the accuracy of GWAS thus the objective of this study was to compare GWAS using 54k Axiom SNP chip and low pass sequencing. For this study shell color was measured as a composite index (with chromometer) and averaged for 3 eggs at approximately 35 weeks of age for 1080 White Leghorn hens. These hens were genotyped with a customized 54k SNP chip, and sequenced at 1x coverage with genomic information reported at 32M locations, 7.6 M of which were segregating with frequency >1%. Additional analysis was performed with 2755 hens added that had chip data and phenotypes but no sequence to verify the signal. PCA analysis did not show any strong stratification, therefore a simple association analysis was performed in Plink, adjusting for first 3 principal components as covariates did not have strong impact on the results. Two strong signals ($P < 10^{-20}$) were detected, one on Chr 9 at 22Mb, and one on Chr 20 at 104-105Mb. The signal co-localized on Chr 9 with MFSD1 and OCX32, LXN, RSRC1, GFM1, MLF1 and SHOX2 of which OCX32 was previously suggested as associated with shellcolor in layers. According to annotation one SNP in SHOX2 was classified as missense mutation with a moderate impact on encoded protein, while other highly significant SNPs had LOW impact or MODIFIER annotation. On Chr20: VSTM2L, CTNBL1, WFDC2, UBE2C, SNX21, TNNC2, ACOT8, ZSWIM3, CTSA, NEURL2, PLTP, and PCIF1, with 13 variants were annotated as having high or moderate impact on encoded proteins, but none of them were among the lowest p-value locations. The signals were detected with the same strength at the same location using both chip and sequence data. Sequence data allowed combining GWAS signal with annotation, however, due to LD it did not narrow down the candidate region. Additional chip data made the signal stronger and narrowed down the region on Chr 20 by 0.4Mb. To take full advantage of sequencing, tens of

thousands of records are needed to break LD, otherwise its benefits for GWAS resolution above carefully designed SNP chip may be limited.

Key Words: GWAS, low-pass sequencing, shell color

28 Reducing susceptibility to pendulous crop in turkey (Meleagris gallopavo) via genomic selection: estimation of genetic parameters, accuracy and bias. Emhimad Abdalla^{*1}, Bayode O. Makanjuola¹, Nienke van Staaveren^{1, 4}, Ryley Vanderhout¹, Ben J. Wood^{1, 3, 5}, Christine F. Baes^{1, 2}; ¹*Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, Ontario, Canada*, ²*Vetsuisse Faculty, University of Bern, Institute of Genetics, Bern, Switzerland*, ³*School of Veterinary Science, University of Queensland, Gatton, Queensland, Australia*, ⁴*The Campbell Centre for the Study of Animal Welfare, University of Guelph, Guelph, Ontario, Canada*, ⁵*Hybrid Turkeys, Kitchener, Ontario, Canada*.

Pendulous crop (PC) in turkeys occurs when the crop distends from its normal position preventing the normal movement of feed and water into the digestive system. This condition negatively impacts production and welfare. In this study, we estimated the genetic parameters for PC incidence and its genetic correlation with five production traits. Additionally, we evaluated the prediction accuracy and bias of breeding values for selection candidates using pedigree (BLUP) or pedigree-genomic (ssGBLUP) relationships among the animals. A total of 170,844 records were made available by Hybrid Turkeys, Kitchener, Canada. Of these, 5,445 were affected with PC. In addition, the data included 9,634 records for breast meat yield (BM); 5,592 records for feed conversion ratio (FCR) and residual feed intake (RFI) in males; 170,844 records for body weight (BW), and walking score (WS) between 18 and 20 weeks of age for males (71,012) and females (99,832), respectively. Within this population, 36,830 animals were genotyped using a 65K SNP chip (Illumina Inc.). All animals passed the quality control criteria, and 53,455 SNPs were retained for subsequent analysis. Heritability for PC was estimated at 0.16 ± 0.01 and 0.17 ± 0.01 using BLUP and ssGBLUP, respectively. The incidence of PC was not genetically correlated with WS or FCR. Low unfavourable genetic correlations with BW (0.12 and 0.14), BM (0.24 and 0.24) and RFI (-0.33 and -0.28) were obtained using BLUP and ssGBLUP, respectively. Using ssGBLUP showed higher prediction accuracy (0.21) for the breeding values for the selection candidates than the pedigree-based model (0.14). Whereas the bias of the prediction was slightly reduced with ssGBLUP (0.33 ± 0.05) than BLUP (0.30 ± 0.08), both models showed a regression coefficient lower than one, indicating inflation in the predictions. These results suggest that PC is a heritable trait and selection for lower PC incidence is feasible. Although further investigation is necessary, selection for BW, BM and RFI may increase PC incidence. Incorporating genomic information would

lead to higher accuracy in predicting the genetic merit of PC incidence in selection candidates.

Key Words: pendulous crop, single-step, genetic correlation, prediction accuracy, prediction bias

29 Single-step genomic evaluation of hatchability trait in turkeys (*Meleagris gallopavo*) using a random regression model. Bayode O. Makanjuola^{*3}, Emhimad Abdalla³, Ben J. Wood^{3,1,2}, Christine F. Baes^{3,4}; ¹*School of Veterinary Science, University of Queensland, Gatton, Queensland, Australia*, ²*Hybrid Turkeys, Kitchener, Ontario, Canada*, ³*Centre for Genomic Improvement of Livestock, Animal Biosciences, University of Guelph, Guelph, Ontario, Canada*, ⁴*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland*.

Hatchability traits are economically important due to their direct effect on poult output. Sustainability and production of turkey poults is a major priority for turkey breeding companies. Hatchability is traditionally recorded as the number of fertile eggs that hatched into poult (HOF) and is collected on a weekly or biweekly basis. This trait is collected throughout the productive life of the animal and is cumulated, thereby resulting in each animal having a single record for the trait. Furthermore, genetic evaluation of this trait has been estimated using pedigree relationships. However, the longitudinal nature of the trait and the availability of genomic information has revived interest in using random regression (RR) to capture the differences in repeatedly recorded traits, as well as the incorporation of genomic relationships. Therefore, the objectives of this study were to compare the applicability of a RR model with a cumulative (CUM) model using both pedigree and genomic information for genetic evaluation of HOF, as well as to estimate expected selection responses. For this study, a total of 63,935 biweekly HOF records from 7,211 hens mated to 1,524 toms were available from a maternal turkey line. In total, 4,832 animals had genotypic records and the pedigree record had 11,191 animals. Estimated heritability from the CUM model was 0.24 for HOF with pedigree information. With RR, heritability estimates ranged from 0.11 to 0.17 for HOF using pedigree relationship. The incorporation of genomic relationships subsequently increased heritability by an average of 28% and 23% for the CUM and RR models, respectively. Expected selection response per year ranged from 1.06 to 5.73 for all studied traits. Estimated responses from RR were more than two fold estimates from the CUM model. Likewise, selection responses estimated using only pedigree information were lower than those including genomic information. Our findings suggest that a RR model using pedigree and genomic relationships caused higher response to selection compared to a CUM model.

Key Words: genomic and pedigree; single-step; random regression; turkeys

30 Tracking vascular inflammation associated with

white striping in broilers using expression profiling.

Michael J. Mienaltowski*, Ubaldo De La Torre, Oona Vanhatalo, Annie J. King; *Animal Science, University of California Davis, Davis, California, United States.*

Fast-growing broilers often demonstrate myopathies like white striping, which negatively impacts meat quality, consumer appeal, and industry earnings. Our primary objective in this study was to discern molecular mechanisms associated with white striping. We hypothesized that gene expression pathways associated with microvascular damage and macrophage activation would initiate ahead of and become more pronounced throughout the progression of white striping in broiler muscle. Commercial Cobb 500 broilers were fed a conventional corn/soy meal diet from hatching to market weight with twenty broilers culled each week. Gross pathology and histology (hematoxylin & eosin, Masson's Trichrome) were documented for pectoralis major muscles. Total RNA was isolated from pectoralis major muscle. For 15 broilers from each week, mRNA was purified, fragmented and converted to cDNA. Adapters were ligated to the ends of double-stranded cDNA and PCR amplified to create libraries. Illumina NovaSeq Sp500 RNA sequencing generated 250 bp paired end reads. Reads were checked for quality with multiQC v1.7, trimmed using trimmomatic PE, and then aligned to the annotated Gallus gallus genome (GRCg6) and quantified using Salmon software. Quantitative files were applied to DESeq2. Contrasts were analyzed using Panther and Advaita Corporation's iPathwayGuide. Weights of culled broilers were analyzed using ANOVA analyses with Tukey's multiple comparison tests. White striping scores, pathology ranks, and histopathology scores of the culled birds were analyzed using Goodman and Kruskal's gamma non-parametric measure of correlation. RNAseq data were analyzed using DESeq2, which performed Wald tests of pairwise comparisons between weeks with Benjamini-Hochberg multiple test corrections. When all broilers were assessed for white striping score, pathology rank, and histopathology score, we found a highly positive correlation with cull weight and week. Pathological changes were evident as early as week two, and transcriptomics revealed changes in expression corresponding to the pathology. By the third week, severity in pathology was evident with expression differences indicative of involvement of pathways associated with natural killer cell mediated cytotoxicity, chemokine signaling, and leukocyte transendothelial migration. Thus, as Commercial Cobb 500 broilers grew, pectoralis major muscle pathology was evident as early as two weeks post-hatching. Vascular inflammation appeared to increase over time and was associated with the severity of muscle pathology. Future efforts should be directed at preventing or reducing vascular inflammation to determine if it is a cause or symptom of the muscle changes.

Key Words: broiler, white striping, pectoralis major, inflammation, transcriptomics

31 A demonstration of amitosis in post-embryonic erythrocytes, possibly due to mycotoxin exposure - a 60-year-old challenge met. Paul Cotter*; *Biology, Framingham State University, Arlington, Massachusetts, United States.*

During development cells of the early erythrocyte series normally divide by a mitotic process. Chromosomal duplication and separation with the aid of a spindle apparatus assure daughter cells receive an equal allotment of genetic material. In rare cases when the division does not involve either chromatin condensation or production of a spindle; it is described as amitosis. Over 60 years ago in his classic treatise, Lucas illustrated an embryonic erythrocyte appearing to be dividing by amitosis. However, he doubted this process could occur in post-embryonic cells. He hypothesized that a firm establishment of amitotic nuclear division might require in vitro study. The objective is to describe the process of amitotic division in post-embryonic erythrocytes. Materials and methods: During a light microscopic study (40x) of ~ 20 duck blood smears per interval (2 to 20 wk) and stained with Wright- Giemsa, dividing atypical cells were identified and photographed (100x). The division process begins with constriction of the nucleus followed by constriction of the cytoplasm. At early stages both the cytoplasm and nuclear constriction continue and the cell begins to separate. At intermediate stages the nascent daughter cells further separate and may remain attached only through an isthmus containing both chromatin and cytoplasm. At the late-stage separation is nearly complete, and daughter cells remain attached only by a thin thread. Chromosomes or a spindle apparatus are never observed in such cells and the nuclear membrane remains intact throughout amitotic division. In rare cases, one daughter cell may again separate producing an erythroplastid, a cell devoid of nuclear material. This process is termed “complex amitosis”. Therefore, amitosis can occur in both normochromic (mature) or in earlier polychromic erythrocyte stages. It is proposed that these observations establish amitosis in post-embryonic circulation. They are sufficient to meet the challenge long-ago proposed by Lucas. Moreover, as the blood samples were obtained from ducklings and older ducks with fungal infection or deliberately exposed to AFB₁, amitosis could result from mycotoxin induced damage to DNA. Therefore, amitosis and erythroplastid production may be useful as cytological indicators useful in the study of mycotoxicosis.

Key Words: amitosis, erythroplastid, erythrocyte, mycotoxin, hematology

32 Synergistic induction of poultry host defense peptides by epigenetic compounds. Melanie Whitmore*, Glenn Zhang; *Animal and Food Sciences, Oklahoma State University, Stillwater, Oklahoma, United States.*

A need for effective antibiotic alternatives is ever increasing, given restricted use of in-feed antibiotics in the U.S. and many other countries. Modulation of endogenous

host defense peptide (HDP) synthesis has emerged as a promising antibiotic-alternative approach. We previously identified a number of epigenetic modifiers to be capable of inducing HDP synthesis following a high-throughput screening. To further assess whether different classes of epigenetic compounds such as histone deacetylase inhibitors (HDACi) and histone methyltransferase inhibitors (HMTi) have a synergistic effect on HDP gene induction, chicken HTC macrophage cells were treated with two different HDACi (butyrate and mocetinostat) and two HMTi (BIX01294 and UNC1999) individually or in combination for 24 h, followed by total RNA extraction and RT-qPCR analysis of chicken HDP gene expression. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Our results indicated that BIX01294 synergized significantly with butyrate or mocetinostat to increase the expression of avian β -defensin 1 (AvBD1), AvBD2, AvBD8, AvBD9, AvBD14, and cathelicidin 2 ($P < 0.05$). A combination of UNC1999 with butyrate or mocetinostat also significantly induced AvBD1, AvBD4, AvBD9, and AvBD10 in a synergistic manner ($P < 0.05$). Chromatin immunoprecipitation (ChIP) was further performed to explore the synergistic mechanism. HTC cells were treated with butyrate and BIX01294 individually or in combination for 12 h, followed by ChIP with the antibody specific to acetylated histone 3 (H3Ac) and qPCR analysis with primers targeting the AvBD9 gene promoter. We found that butyrate and BIX01294 synergistically enhanced H3Ac acetylation at the AvBD9 gene promoter ($P < 0.05$). Taken together, our results indicated that HDACi and HMTi cooperate with each other to induce HDP gene expression through promoting histone acetylation of HDP gene promoters, which likely results in chromatin relaxation and increased accessibility of gene promoters to transcription factors. The outcomes of the research could potentially lead to the development of epigenetic modifiers as novel alternatives to antibiotics for disease control and prevention in poultry and likely other livestock species as well.

Key Words: host defense peptides, antibiotic alternatives, epigenetic regulation, histone acetylation

33 Expression characteristics of chicken lncRNA FKBP5-AS and its effect on proliferation and differentiation of myoblasts. Pengfei Du*^{GS}, Xiangli Zhang, Ziyang Wang, H.Y Zhang, Y.Q. Huang, W Chen; *College of Animal Science and Technology, Henan Agricultural University, Zhengzhou, China.*

Long noncoding RNAs (lncRNAs) have recently gained considerable attention as key players in growth regulation of livestock. However, the role of lncRNA in the regulation of chicken skeletal muscle development remains poorly understood. LncRNA FKBP5-AS has been identified based on our transcriptome data, in this research, the expression characteristics of lncRNA FKBP5-AS and its effect on proliferation and differentiation of chicken myoblasts were further investigated. qRT-PCR was used to detect the

expression levels of FKBP5-AS in different tissues of Arbor Acres (AA) broilers at different developmental stages (E12, E19, D21, D42). 24 d male AA broilers were injected intraperitoneally with insulin (5 IU/kg) in the experimental group and phosphate buffered saline in the control group (n=8). 21 d male AA broilers were randomly divided into three groups and received intraperitoneal injection of glucose solution (2g/kg), sodium pyruvate (2g/kg) or saline (n=8). Twenty 18-day-old female broilers were randomly divided into ad libitum group (AL) and energy restriction (ER) group. From 18~48 d, AL broilers were fed ad libitum with control diet (13.17 MJ/kg ME, according to CFSC, 2004), ER broiler was subjected to 30% energy restriction. Data were analyzed by one-way analysis of variance with a Duncan's multiple-range test. The results showed that FKBP5-AS had the highest expression level in skeletal muscle, especially in embryonic (E19) pectoral muscle ($P < 0.01$). Responded to exogenous insulin, glucose, sodium pyruvate and 30 % energy restriction, the expression of FKBP5-AS was significantly increased after 120 min of insulin treatment, 10 min of glucose treatment and 60 min of sodium pyruvate treatment ($P < 0.01$), and the expression of FKBP5-AS was decreased after 30 % energy restriction ($P < 0.05$) when compared with the control group. Furthermore, after knocking down FKBP5-AS in chicken myoblasts using small interfering RNA, the proliferation key genes including cyclin-dependent kinase 2 (CDK2), proliferating cell nuclear antigen (PCNA), and Cyclin D1 (CyclinD1) were significantly upregulated ($P < 0.05$), which was confirmed by increased cell activity from cell counting assay ($P < 0.01$). Knocking down FKBP5-AS also resulted in decreased the expression level of the differentiation key gene such as myogenin (MYOG) and myosin heavy chain (MYHC) ($P < 0.01$). In conclusion, the novel identified lncRNA FKBP5-AS were mainly expressed in skeletal muscle, and inhibited proliferation and promoted differentiation of chicken primary myoblasts. These results will shed a light for further exploring the physiological function and regulation mechanism of chicken FKBP5-AS.

Key Words: FKBP5-AS, myoblasts, proliferation, differentiation, chicken

34 Identification and functional study of insulin-responsive microRNAs in chicken pectoralis. Xiangli Zhang*^{GS}, Pengfei Du, H.Y Zhang, B. H. Shao, Ziyang Wang, Yongshuai Wang, Y.Q. Huang, W Chen; *College of Animal Science and Technology, Henan Agricultural University, Zhengzhou, Henan, China.*

Although emerging evidences indicated that microRNA (miRNA) is of considerable significance in both development and metabolism, the role of insulin-responsive miRNAs in the development and metabolism of skeletal muscle remains poorly understood in chicken. Here, the miRNAs related insulin-responsive were identified in chicken pectoralis. With this aim, forty 24-day-old male Arbor Acres broilers were randomly divided into two groups and received intraperitoneal injection of 5 IU/kg

insulin or phosphate buffered saline (PBS) after 16 h of fasting. Blood glucose levels were measured and pectoralis was collected for RNA sequencing at 15 min and 120 min after injection. Data all were analyzed by one-way analysis of variance with Duncan's multiple-range test. Results showed that blood glucose gradually declined and reached to minimum at 120 min ($P < 0.01$) as insulin treatment. Furthermore, we screened 616 known miRNAs and 66 novel miRNAs of pectoralis through RNA sequencing; Compared with the PBS group, 18 and 14 differentially expressed miRNAs (DEMs) were screened after insulin injection for 15 and 120 min, respectively; 31 DEMs were screened after insulin injection 120 min relative to 15 min ($P < 0.05$ and $|FC| \geq 1$). The outcomes from GO and KEGG enrichment analysis on the target genes of DEMs showed that target genes mainly enriched in glucose metabolism and muscle development. Of note, miRNA-214b-3p was observed remarkably down-regulated after insulin treatment for 120 min and during the period from 15 to 120 min. Accordingly, the expression characteristics of miR-214b-3p in chicken myoblast was further defined, and the data showed that the expression of miR-214b-3p in the proliferation stage was higher than that in differentiation stage ($P < 0.01$). We detected the effect of miR-214b-3p overexpression on the proliferation of chicken myoblast using CCK8. Subsequently, we overexpressed miR-214b-3p for 48 h and stimulated chicken myoblast with 0.02 μ M insulin for 20 min, collected the cells and detected the expression of related marker genes by qPCR. Results shown that miR-214b-3p overexpression could promote cell proliferation and inhibit cell differentiation of chicken myoblasts ($P < 0.05$). MiR-214b-3p also could inhibit the glycogenesis of chicken myoblasts but promote insulin signal transduction ($P < 0.05$). In summary, the current study shows that certain miRNAs are modified response to insulin manipulation, especially miR-214b-3p that plays a key role in the glucose metabolism of chicken myoblasts, proposing them as potential resource for further studies on the specific mechanisms of miRNA in skeletal muscle development and glucose metabolism in chickens.

Key Words: insulin, chicken, miRNA, skeletal muscle, glucose metabolism

35 Effect of probiotics on tight junction protein mRNA in the small intestine of young chicks. Meiting Jia*^{GS}, Eric Wong; *Virginia Tech, Blacksburg, Virginia, United States.*

Tight junction (TJ) proteins in the small intestine form a paracellular barrier to prevent intestinal pathogens from entering the blood circulation and to regulate the permeability of ions, water and solutes. The objective of this study was to investigate the effect of probiotics on the mRNA abundance for the TJ proteins claudin 1 (CLDN1), occludin (OCLN), zonula occludens 1 (ZO1) and junctional adhesion molecule 2 (JAM2) in the small intestine of young broiler chickens. Cobb 500 broiler chicks (n = 204) were hatched and randomly placed into 3 treatments: basal diet

without probiotics (Control) and two basal diets supplemented with different probiotics (Probiotic 1 or Probiotic 2). Each treatment had 6 replicate cages. At day (d) 2, d4, and d6, one chick from each replicate cage was randomly selected for collection of the duodenum, jejunum, and ileum. A piece from the middle of each segment was fixed for paraffin embedding and in situ hybridization analysis. An additional piece was minced and stored at -80 for gene expression analysis. Two-way ANOVA and Tukey's test were used for statistical analysis. Significance was set at $P < 0.05$. Probiotic 2 significantly increased CLDN1 mRNA in the ileum compared to Control ($P < 0.05$). There were no differences in the mRNA abundance of the other TJ proteins, and no significant interactions between treatment and age for mRNA expression of all the selected TJ proteins. In situ hybridization showed that OCLN mRNA was expressed in the epithelial cells of both the villus and crypt in all three small intestinal segments. There was greater intensity of the OCLN signal in the bottom of the villi compared to the tip of villi on day of hatch. These results suggest that Probiotic 2 may improve intestinal integrity in the ileum by increasing CLDN1 mRNA expression.

Key Words: tight junction proteins, probiotics, small intestine, in situ hybridization, chicken

36 Effect of fasting and heat stress on blood parameters, carcass and meat quality of two broiler chicken strains. Demilade I. Ibiwoye*^{GS 1, 2}, Bisola Sule¹, Foluke E. Sola-Ojo¹; ¹*Department of Animal Production, University of Ilorin, Ilorin, Kwara, Nigeria,* ²*Key Laboratory of Agricultural Animal Genetics Breeding and Reproduction of Ministry of Education, Huazhong Agricultural University, Wuhan, Hubei, China.*

Exposure to high ambient temperatures has long been recognized as having a detrimental effect on broiler production efficiency and meat yield. Seasonal heat exposure has been reported to cause undesirable changes in meat characteristics. The main purpose of this experiment was to show the effect of different management heat stress, fasting and heat stress of both persistent (gradual/daily) and acute (sudden) on carcass trait, blood parameters of two genotype of broilers (Arbor acre and Hubbard). Use of unsuitable genotypes of birds in hot regions results in decreased growth rate, reduced protein gain and high mortality. A total of 800 (one day old chick) with equal numbers of Hubbard and Arbor Acre was used for this study. The birds were randomly distributed into 8 experimental group comprising of both genotypes and thereby grouped into 4 groups for each type of heat stress per genotype (Fasting and Acute Heat Stress) for a period ranging from 0 min to 90 minutes feed restriction. The above regime was also followed for the Fasting and Persistent Heat Stress group. For the Meat quality analysis, a total of 80 adult broilers of both genotypes were used for the study. The Carcass traits were measured and meat quality was determined with the birds of both genotypes to

know the genotype that has better carcass and meat quality attributes. The result obtained from this study shows that the effect of carcass trait on heat stress (acute and persistent) and feed restriction on Hubbard and Arbor Acre. The result shows that there is significant different ($p < 0.05$) in dressing percentage of persistent heat stress, dressing weight and dressing percentage of Hubbard. Also, drip loss for Arbor Acre strains is significant in both persistent and acute conditions. Consistently, cooking loss for Hubbard strain is higher and has more quality of water lost than Arbor acre strain and persistent bird had higher cooking loss than acute heat stress. It was thereby concluded from the study that Arbor acre has a good and higher carcass quality than Hubbard and the strain is preferred to be used by farmers in the hot seasons of the year.

Key Words: broiler chickens, strain, heat stress, cooking loss, drip loss

37 Effects of chronic heat stress on the expression of nutrient transporters in the jejunum of modern broilers and their ancestor Wild Jungle Fowl. Nedra Abdelli*^{GS 1}, Alison Ferver³, Sara Orłowski⁴, Travis Tabler², Elizabeth S. Greene⁵, Sami Dridi²; ¹*Animal and Food Science Department, Autonomous University of Barcelona, Sabadell, Barcelona, Spain,* ²*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States,* ³*Cell and Molecular Biology, University of Arkansas, Fayetteville, Arkansas, United States,* ⁴*Poultry Science, University of Arkansas, Springdale, Arkansas, United States,* ⁵*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States.*

Heat stress (HS) is one of the main threats to poultry production sustainability via its strong adverse effects on broiler growth, feed efficiency, meat yield, and welfare. The compromised growth performance maybe attributed to leaky gut syndrome and a change in nutrient absorption. This study aims to evaluate the effect of chronic HS on the expression of nutrient transporters in the jejunum of 4 populations: Athens Canadian Random Bred (ACRB), 1995 Random Bred (95RAN), 2015 Random Bred (MRB), and Jungle fowl (JF). One-day male chicks ($n=150$ /line) were placed by line in environmentally controlled chambers. A daily cyclic heat stress (36 degrees Celsius) was applied to half of the chambers on day 28 (HS) until the end of the trial; the remaining chambers remained under thermal neutrality (TN) at 24 degrees Celsius. Growth performance was evaluated and jejunum tissue was collected on day 54. The gene expression of carbohydrate transporters Glut1, Glut2, Glut5, Glut6, Glut8, Glut9, Glut10, Glut11, Glut12, Glut14 and SLC5A1; amino acid transporters LAT1, SLC6A14, SLC7A1 and SLC7A6 and fatty acid transporters CD36 and iFABP was determined by qPCR. Western blot analysis of some of the aforementioned proteins was also conducted. Growth performance and gene expression data were analyzed using two-way ANOVA considering chicken population and environmental condition as the main effects. If ANOVA showed

significant effects, the means were compared by Tukey's multiple range test using GraphPad Prism 6.00; significance was set at $P \leq 0.05$. Under both TN and HS conditions, MRB showed the highest BW54 and cumulative FI followed by the 95RAN. The cumulative FCR had a line effect ($P < 0.0001$), however, no environmental treatment effect was observed ($P = 0.8185$). Under HS, JF showed higher gene expression of carbohydrate (Glut1, Glut5, Glut8, Glut9, Glut10 and SLC5A), amino acids (SLC6A14 and SLC7A6) and fatty acid (CD36 and iFABP) transporters compared to the other populations. HS upregulated the expression of Glut1, Glut5, Glut10, SLC6A14, CD36 and iFABP in JF, and reduced Glut2 and Glut10 in MRB. Preliminary results from the western blot showed a decrease of Glut3 protein expression in all lines under chronic HS. Together, these data showed: 1) a population-dependent expression of nutrient transporters, which may explain the difference in feed efficiency between the chicken population, and 2) that HS alters the expression of these transporters.

Key Words: heat stress, broiler, gene expression, protein expression, nutrient transport

38 Evaluation of Olfm4 and Muc2 expression in the jejunum of chickens divergently selected for eight week body weight. Sydney Kinstler*^{GS}, Sara Cloft, Paul Siegel, Christa Honaker, Eric Wong; *Virginia Polytechnic Institute and State University, Christiansburg, Virginia, United States.*

Divergent lines of White Plymouth Rocks have been selected for high weight (HWS) and low weight (LWS) for over 63 generations with the LWS line developing anorexia nervosa causing starvation even with ad libitum access to feed. Symptoms of starvation in the LWS chicks, include failure to provide nutrition to organs and intestines and inhibition of the development of absorptive capabilities and immune function in the gut, which increase mortality risk. Nutrient deprivation is known to affect intestinal function and the regeneration of the mucosa layer as well as epithelial turnover, but these pathways are not well understood in the LWS line. The objective of this study was to determine the effect of nutrient deprivation on intestinal stem cell proliferation and mucus production in LWS birds in the post-hatch period. Jejunal samples from 6 HWS and 6 LWS birds were collected on day of hatch (doh) and day (d) 3 post-hatch for histology analysis. Samples were embedded in paraffin and analyzed using the RNAscope in-situ hybridization method to examine expression of mucin 2 (Muc2), an important mucus protein that is expressed in goblet cells and olfactomedin 4 (Olfm4), a stem cell marker. Images were captured under 40x magnification for localization and enumeration of Olfm4 and Muc2 expressing cells and villus morphology. Villus height (VH), crypt depth (CD), and VH/CD were measured for the Olfm4 images using ImageJ. Muc2 images were analyzed to determine the density of goblet cells. One-way ANOVA was performed for all measurements. Goblet cell density

was greater in HWS birds than LWS birds on doh ($P < 0.0001$). From doh to d3, goblet cell density increased in HWS birds ($P < 0.0001$), but decreased in LWS birds from doh to d3 ($P < 0.0001$). VH, CD, and VH/CD was similar between LWS and HWS birds ($P > 0.05$) on doh, but was less in LWS birds than HWS birds on d3 ($P < 0.0001$). Olfm4 expressing stem cells were similar between LWS and HWS birds on doh, but increased in HWS birds and decreased in LWS birds from doh to d3. These results show that from doh to d3 HWS birds increased stem and goblet cells, but LWS birds decreased stem and goblet cells, which may partially explain their impaired growth characteristics.

Key Words: goblet cells, intestinal stem cells, Muc2, Olfm4, selected lines

39 Genome-wide SNPs regulating nervous system functions associated with stress response traits in high and low stress lines of Japanese Quail. Steven A. Shumaker*^{GS1}, Bhuwan Khatri², Dongwon Seo³, Stephanie Shouse¹, Seong W. Kang¹, Wayne J. Kuenzel¹, Byungwhi Kong¹; ¹*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*, ²*Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, United States*, ³*Animal Science, Chungnam National University, Daejeon, Korea (the Republic of).*

Stress management is an important part of maintaining production efficiency in poultry and improving management techniques is reliant on our understanding of physiological stress responses. The nervous and neuroendocrine systems play important roles in these stress responses. Single Nucleotide Polymorphisms (SNPs), common genetic mutations responsible for genotypic and phenotypic diversity in animals, can be used as biomarkers for detection of genetic alterations. As part of an analysis to understand the genetic mechanisms underlying stress responses, whole genomic resequencing was performed on 20 birds from High Stress (HS) and Low Stress (LS) lines of Japanese quail using Illumina HiSeq 2 x 150 bp paired end read technology to detect SNPs within the two lines of quail. Alignment of sequencing data to the quail genome found 6,364,907 SNPs across both lines with 10,364 SNPs occurring in coding regions. Of these SNPs, we identified 2,886 unique, non-synonymous SNPs with a SNP% ≥ 0.90 and a read depth ≥ 10 . Ingenuity Pathway Analysis was used to identify significantly affected molecular pathways, functions, and genes with ties to neural development, neurological signaling, and the neuroendocrine system using a Fisher's Exact Test (p -value < 0.05). These findings suggest that the SNPs identified in these two lines of quail could be responsible for observed phenotypic differences in stress responses.

Key Words: stress response, SNPs, quail, nervous system, Ingenuity Pathway Analysis

40 SNP-based breeding for broiler resistance to ascites and evaluation of correlated production traits.

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This study evaluated Marker-Assisted Selection (MAS) for ascites resistance in broilers, assessing its impact on disease phenotype and related production traits. Previously, we used whole genome resequencing (WGR) to fine-map 28 chromosomal regions as associated with ascites phenotype. SNP genotypes were determined and used to selectively breed 2 generations using our experimental ascites broiler line (REL) to produce birds homozygous for the resistant chromosomal alleles. The F2 generation was then compared to the REL line for ascites phenotype and production traits. For ascites phenotype, 2 cohorts of MAS and REL mixed-sex offspring were placed in a hypobaric chamber for 5 wk each. Cohort 1 (T1; n=332 MAS, 246 REL) was maintained at 9,000 ft simulated elevation; Cohort 4 (T4; n=212 MAS, 221 REL) was maintained at 11,000 ft. Mortalities were necropsied for BW, fluid presence, heart condition, and right ventricle to total ventricular (RVTV) ratio. For production traits, 2 cohorts of MAS and REL mixed-sex offspring (T2: n=233 MAS, 220 REL; T3: n=206 MAS, 209 REL) were placed in floor pens for 8 wk each and fed an industry-standard diet in starter (0 to 14 d), finisher (14 to 35 d), and withdrawal (35 to 56 d) phases. Pen BW was taken at 0, 2, 4, 6, and 7 wk. Feed conversion ratio (FCR) was calculated based on the final wk of growth and all birds were processed at 8 wk. For meat quality, the deboned breasts of 200 birds (100 MAS/REL: 50male/female) were evaluated for color, pH, drip and cook loss, and shear force. Additionally, 100 birds (50 MAS/REL: 25 male/female) were assessed on heart, liver, lung, and spleen weights, and RVTV ratio. Ascites phenotype and processing, organ weight, meat quality data were analyzed by 2-way ANOVA and live production data analyzed by 1- way ANOVA. All means were separated by Tukey's HSD ($P \leq 0.05$). MAS resulted in sex- and simulated elevation- dependent reduction in ascites incidence in both cohorts. Compared to the REL line, T1 MAS saw a decrease ($P=0.041$) in ascites incidence while T4 MAS saw a numerical decrease ($P=0.162$) in ascites incidence. Comparisons of the MAS and REL lines found no negative ($P > 0.05$) impacts on the MAS line in feed intake, FCR, deboned parts, or meat quality. There were some improvements in single cohorts of MAS, but the only improvements consistent in both cohorts were for absolute and relative tenders weights ($P \leq 0.014$). We conclude that the MAS line is comparable and, in some instances, superior, in growth performance to the control while being more resistant to ascites. This study represents the first SNP-based selection program for a complex trait and indicates that MAS based on WGR has significant breeding potential in agricultural systems.

Key Words: ascites, SNP, breeding, genetics, broiler

41 Expression of genes in the apoptosis pathway in the yolk sac of embryonic and early-post-hatch broiler chickens. Kaitlyn Reno*^{UG}, Sara Cloft, Eric Wong; *Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, Virginia, United States.*

The yolk sac (YS) of an embryonic chick starts to be internalized and degrades during late embryogenesis. Apoptosis, also known as programmed cell-death, is speculated to be part of this degradation process. There are many proteins involved in apoptosis, but the primary ones are initiator and executioner caspases. Initiator caspases are among the first apoptotic enzymes to be activated and their role is to activate the executioner caspases and cytochrome C which disrupts the integrity of the cell and destroys proteins in the cell. The objective of this study was to examine the mRNA abundance of genes involved in the apoptosis pathway in the YS of Cobb 500 chickens at embryonic day 17 (e17), e19, day of hatch (doh), and day (d) 1.5, d3, d5 and d7 post-hatch. The genes investigated in this study included, the apoptosis inhibitor Bcl2, the initiator caspases 8 and 9, the executioner caspases 3, 6, and 7, and cytochrome c. RT-qPCR was performed to analyze gene expression using the e17 YS sample as the calibrator. Statistical analysis using least squares means contrasts in JMP was performed on the gene expression data to determine if there was a linear, quadratic, or cubic contrast. The results showed a significant linear response for caspases 3 ($P = 0.0022$), 7 ($P = 0.0039$) and 8 ($P = 0.0061$), cytochrome c ($P = 0.0004$) and Bcl-2 ($P = 0.0017$) from e17 to d7. Caspase 9 had a significant quadratic contrast ($P < 0.0001$), i.e., an increase from e17 to d1.5 and then a decrease to d7. Caspase 6 had significant linear ($P = 0.0023$) and quadratic ($P = 0.0026$) contrasts. In summary, a number of caspase genes that are involved in the apoptosis pathway are upregulated in the YS from e17 to d7. However, the anti-apoptotic gene Bcl-2 is also upregulated, which suggests that degradation may be occurring through a non-apoptosis pathway.

Key Words: yolk sac, apoptosis, caspase, broiler, Bcl-2

42 L-Tryptophan and phosphate sensing by the chicken extracellular calcium-sensing receptor via potential docking sites to initiate downstream G protein-coupled signaling cascades. Qianru Hui*^{GS 1}, Huanhuan Dong², Xiaoya Zhao¹, Paula Azevedo¹, Martin Nyachoti¹, Karmin O^{3, 1}, Chengbo Yang¹; ¹*Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada*, ²*Jiangxi University of Chinese Medicine, Nanchang, Jiangxi, China*, ³*CCARM, St. Boniface Hospital Research Centre, Winnipeg, Manitoba, Canada*.

Laying hens have a dynamic bone turnover and a high demand for calcium from both feed and bone storage due to the daily egg-laying cycle. This study aims to reveal the modulation mechanism and binding sites of an agonist (L-tryptophan) and an antagonist (inorganic PO_4^{3-}) to chicken extracellular calcium-sensing receptor (cCaSR). Based on a

luciferase reporter system (in response to the second messengers), the two modulators showed biased agonism or antagonism through $G\alpha_q$, $G\beta\gamma/G\alpha_i$, and $G\alpha_{12}$ -mediated signaling pathways and induced the changes in the second messengers, including intracellular Ca^{2+} , ERK1/2, and RhoA, respectively. Each well from a multi-well plate was used as the experimental unit for all analyses. All data were expressed as mean \pm 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. L-tryptophan-induced cCaSR activation required the presence of Ca^{2+} , and the Ca^{2+} concentration affected its potency. The EC_{50} curve for Ca^{2+} left-shifted by 13.57%, 5.18%, and 1.02% in the presence of 1 mM L-Trp in the $G\alpha_q$, $G\beta\gamma/G\alpha_i$, and $G\alpha_{12}$ -mediated signaling pathway. The cCaSR response was significantly inhibited with increasing PO_4^{3-} concentration by 92.97%, 31.50%, and 30.23% at 2 mM PO_4^{3-} compared with the control in the $G\alpha_q$, $G\beta\gamma/G\alpha_i$, and $G\alpha_{12}$ -mediated signaling pathway. Therefore, both L-tryptophan and PO_4^{3-} biased to $G\alpha_q$ -mediated NFAT-RE

signaling pathway. According to molecular docking results, there was one predicted binding site of L-tryptophan to cCaSR with the residue interaction at Trp 70, Ser 147, Ala 168, Ser 169, Ser 170, Tyr 218, Ser 272, Glu 297, and Ala 298. There were two predicted binding sites of PO_4^{3-} to cCaSR. The first binding site interacted with residue Arg 66, Arg 69, His 412, Arg 414, and Ile 415; the second binding site interacted with residue His 192, Thr 195, Lys 225, Lys 516, and Arg 519. In conclusion, this study demonstrated that similar to human CaSR, L-tryptophan and inorganic PO_4^{3-} were also cCaSR modulators, acting as positive and negative allosteric modulators, respectively, with biased agonism and antagonism *in vitro*. As human CaSR has been recognized as a therapeutic target in osteoporosis, the results also provided theoretical evidence that L-tryptophan and inorganic PO_4^{3-} could be used as potential nutritional ligands to prevent or attenuate chicken osteoporosis through the cCaSR modulation.

Key Words: extracellular calcium-sensing receptor, L-tryptophan, Inorganic phosphate, G protein-coupled signaling, Molecular docking

Immunology, Health and Disease

43 Protective immunity elicited by live commercial coccidia vaccines (LCV) against recent field isolates and vaccine strains. Jennifer R. Timmons^{*1}, Celia Whyte¹, Steve Fitz-Coy³, Samuel N. Mwangi²; ¹*Agriculture, University of Maryland Eastern Shore, Princess Anne, Maryland, United States*, ²*Alcorn State University, Lorman, Mississippi, United States*, ³*Merck Animal Health, Millsboro, Delaware, United States*.

Coccidiosis is a parasitic disease of the intestinal tract of animals caused by coccidian protozoa of the genus *Eimeria*. It is a costly disease associated with poultry production across the world. Use of live vaccines have proven to be important for control of coccidiosis, however vaccines from different companies can vary in terms of number and type of *Eimeria* species, total number of oocyst and attenuated status of the organism. The objectives of this study were to measure the level of protection provided by five live commercial coccidian vaccines (LCV) against homologous and heterologous challenges at 19, 26 and 33 days of age and measure the level of pathology for each isolate in coccidia naïve chickens via histopathology. A Randomized Complete Block design with six treatments and two replicates/treatment (400 birds/replicate). Significant differences among treatments means were determined using Tukey's-HSD test with a 5% level of probability. The dependent variables that were measured were body weight gain and microscopic lesion scores (MS). Four thousand eight hundred chickens were assigned to the following six groups (Non-vaccinated control (Con), vaccine A, vaccine B, vaccine C, vaccine D, and vaccination E). Vaccination was performed via coarse spray (d0). Birds were challenged on 19, 26, and 33 d of age with 3-5 field isolates and a homologous antigen. Four to five birds/replicate were challenged with each field isolate and homologous antigen. MS were determined 6 days post challenge. No differences ($P > 0.05$) were detected in the d 19 to 26 average gain of broilers between the treatments. However, differences were detected in the d 26-32 and d 33-39 average gain of broilers. The average gain of the C treated broilers from d 26-32 and d 33-39 was lower ($P \leq 0.05$) compared to the gain of broilers from the vaccinated treatments. Birds vaccinated with vaccines A, C, and E had a 53.3, 66.7, and 66.7% reduction, respectively in MS when birds were challenged with *E. acervulina* at 19 d of age compared to the Con group that was challenged. Birds vaccinated with vaccine A, C, D, and E had a 70.8, 87.5, 83.3, and 91.7% reduction, respectively in MS when birds were challenged with *E. tenella* at 19 d of age compared to the Con group. Only birds vaccinated with vaccine A demonstrated a MS reduction (66.7%) compared to the Con when these groups were challenged with *E. maxima* at 19 d of age. However, vaccine A, B, D, and E had 69.6, 40, 48 and 89.6% MS reduction, respectively compared to the Con when birds were challenged with *E. maxima* at 26 d of age. Therefore, the

average gain of broilers may be improved when LCV are used to control coccidiosis, and LCV can reduce MS of coccidiosis challenged birds.

Key Words: coccidiosis, broilers, live coccidia vaccine

44 Prevalence of *Eimeria* lesions quantified by ISI application in commercial farms of broiler in South America. Bruna L. Belote^{*1, 2}, Igor Soares¹, Adrien W. Sanches¹, Elizabeth Santin^{1, 2}; ¹*Department of Veterinary Science, Federal University of Parana, Curitiba, Brazil*, ²*ISI Institute, Curitiba, Brazil*.

A continuous and detailed sanitary monitoring is a key tool to insure the effectiveness of antimicrobial programs, mainly when we have plenty of new phyto-genic alternatives being evaluated to replace antibiotics in the birds feed. Therefore, we have developed the ISI application, through which we turn the bird's overall health into numbers in real time. The service is based on the scoring of certain alterations in the respiratory and locomotive systems, and in the gastrointestinal tract of the necropsied birds. Each parameter is scored from zero to 3, according to the extension and intensity of the alteration. The surveyed scores (S) are multiplied by the parameter impact factor (IF), which varies from 1 to 3 according to the impact of that alteration on the tissue physiology. The final ISI score is assessed by the formula $ISI = \sum (IF * S)$, which refers to the sum of all parameters. The present study aimed to survey the prevalence of coccidiosis in broilers farms of South America by using the ISI mobile app. For that, the frequency (%) of macroscopic lesions caused by *Eimeria acervulina*, *E. maxima* and *E. tenella* was assessed in the period of January 1st to December 31st of 2020, with a total 6,130 flocks of broilers evaluated. Five birds between 25 and 35 days of age were euthanized and evaluated per flock, totaling 30,656 animals. Based on postmortem lesions, 5.3 % birds presented lesions of *E. acervulina*, 5.2 % birds presented lesions of *E. maxima* and 5% birds presented lesions of *E. tenella*. The month with the highest frequency of coccidiosis was september (17.8%). Other alterations were frequent in the same period, as respiratory lesions, small intestine lesions, yolk retention and pancreas atrophic. The change of season, variation in grain quality, and poor management could be some of factors associated to it. It is estimated that the lesions provoked by *Eimeria* resulted in a loss of US\$ 0.005 per bird. Nowadays, around 7 billion of broilers are slaughtered per year in South America. Considering that approximately 5% of the evaluated birds presented coccidiosis, irrespective of the *Eimeria* specie, the impact of coccidiosis could reach 350 million dollars a year. Our data reinforces the temporal variation in the prevalence of coccidiosis in broilers flocks, providing an estimative of the economic impact of the disease. This ISI application provides quantitative data in real-time, allowing a agile

decision making and providing possible interactions among the environmental and management factors and the animal health.

Key Words: I See Inside, coccidiosis, gut health, Index, Scoring method

45 Evaluation of anticoccidial capacities of a novel blend of botanicals on *Eimeria*-challenged broilers. Bertrand Medina*, Ashley Wagner¹, Dana Kumprechtova², Ivan D. Girard¹; ¹Probiotech International Inc., St-Hyacinthe, Quebec, Canada, ²Institute of Animal Science, Prague, Czechia.

Coccidiosis can cost around 5.2 US cents/bird and 81% of this comes from lost performances. Although many coccidiostats still exist, poultry industry is always looking for new molecules that may alternate into their existing coccidia-preventive programs to ensure continuous efficacy of current treatments over time. This trial evaluated a novel proprietary feed additive [SYNBIOTEC (SYN), a blend of essential oils and solid plant extracts) designed by Probiotech Int. to support broiler performances under *Eimeria*-challenged conditions. A total of 720 day-old ROSS 308 chicks (36 males/pen, 4 replicates) were randomly allotted into 5 treatments: Non-treated (NT); Monensin at 125 ppm (MON); 500, 375 and 250 ppm of SYN in starter (d1-14, S), grower (d14-28, G) and finisher (d29-42, F) diets (SYN1); 250, 500 and 250 ppm of SYN in S, G and F diets (SYN2) and 500, 250 and 125 ppm of SYN in S, G and F diets (SYN3). At d9, a total 1.4×10^5 attenuated oocysts of *E. acervulina*, *E. tenella* and *E. maxima* (Livacox®, Biofarm) were orally inoculated. Diets were offered in pellet form (crumbles for starter) with no antibiotic growth promoters. Body weight (BW), feed intake, feed efficiency (FCR) and mortality were monitored during all phases. Feces were collected for oocyst counts (OPG) and birds sacrificed (6 birds/pen/d) to assess *E. acervulina* (d13) and *E. tenella* (d14) lesion scores at 4, 5 days post infection (dpi), respectively. Treatment means were compared with a multi-factorial procedure ANOVA and means compared with a Duncan's LSD test. Cumulative mortality was low (<2,1%) and no treatment effect was noticed. At d15, peak of oocyst excretion (6 dpi), only MON reduced the counts (1725 OPG, $P < 0.05$) compared to other treatments. At d21 (12 dpi) MON remained the lowest (1308 OPG, $P < 0.05$) but SYN groups (8883, 10817 and 12575 OPG, for SYN1,2 and 3) did not present any new peak of excretion compared to NT (25 283 OPG, NS). MON birds had also the lowest intestinal lesion scores ($P < 0,05$) compared to other ones, but at 4 dpi, SYN1 (0.45) and SYN3 (0.58) lowered ($P < 0.01$) the lesion due to *E. acervulina* compared to NT (1.13) and at 5 dpi SYN1 (1.0), SYN 2 (0.98) and SYN3 (1.0) lowered ($P < 0.01$) the lesion due to *E. Tenella* compared to NT (1.83). The same treatment-dependent patterns ($P < 0.05$) were observed for BW and FCR values, respectively with the greatest (2.76 kg, 1.73) for MON, the worst (2,23 kg, 1.94) for NT, and intermediate ones (2.43 kg 1.82) for SYN1, (2,40 kg, 1.81)

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for SYN2 and (2.35, 1.82) for SYN3. This novel blend of botanicals seems to be beneficial to preserve growth performances on *Eimeria*-challenged broilers but a minimum of 500 ppm has to be applied to reduce *E. acervulina* intestinal lesions 6 dpi.

Key Words: broilers, botanicals, anticoccidial, intestinal integrity, *Eimeria*-challenge

46 *Bacillus subtilis* based NK lysin peptide delivery system successfully mitigate avian coccidiosis by enhancing the growth and gut health of the broiler chickens. Samiru S. Wickramasuriya*¹, Inkyung Park¹, Jolieke v. Oosterwijk², Chris Przybyszewski², Cyril G. Gay¹, Hyun Lillehoj¹; ¹USDA-Agricultural Research Service, Beltsville, Maryland, United States, ²US Biologic, Inc., Memphis, Tennessee, United States.

Recent studies reported that chicken NK-lysin peptide 2 (cNK-2) has a direct cytotoxicity against apicomplexan parasites such as *Eimeria*. Moreover, cNK-2 modulates gut immune responses to decrease local inflammation triggered by parasite invasion of host cells. A novel oral delivery strategy to express cNK-2 in the intestine where *Eimeria* parasites interact with the host's gut epithelial cells reduce the fecundity of parasites and gut damage. Hence, we developed a stable *Bacillus subtilis*-based NK lysin peptide delivery system to determine its effectiveness as an oral carrier of NK lysin to the gut and to investigate its effect against coccidiosis in commercial broiler chickens. One hundred broiler chickens were allocated into four treatment groups in a completely randomized design: 1) negative control (NC, non-challenged), 2) positive control (PC: challenged without *B. subtilis*), 3) *B. subtilis* with empty vector (EV), and 4) *B. subtilis*-cNK2 (NK). All birds were challenged with 5,000 sporulated *E. acervulina* oocysts through oral gavage except the NC group on day 15. Chickens given *B. subtilis* (EV or NK) were orally gavaged daily from day 14 to day 18 (1×10^{12} cfu/mL). Body weights were measured on 6 and 9 days post-infection (dpi). Duodenum samples were collected on 6 dpi to assess the tight junction and mucosal cytokine gene expressions. Fecal samples were collected from 6 to 9 dpi to enumerate the oocyst shedding. Collected data were analyzed using general linear model procedure of one-way ANOVA of SPSS software. Infected chickens treated with NK showed improved ($p < 0.05$) weight gain, higher ($p < 0.05$) tight junction gene expression, and lower cytokine ($p < 0.05$) gene expression compared to infected and untreated controls. Fecal oocyst shedding was reduced ($p < 0.05$) in the infected chickens treated with NK compared to the infected chickens without NK. In conclusion, this study demonstrates that dietary *B. subtilis* spores carrying chicken NK-2 peptide is a successive strategy to reduce harmful effects of avian coccidiosis in commercial broilers.

Key Words: NK-lysin, Chicken, *Bacillus subtilis*, Coccidiosis, Gut health

47 Optimization of *Clostridium perfringens*

sporulation vaccine to reduce chicken necrotic enteritis. Ying Fu^{*1, 2}, Mohit Bansal², Ayidh Almansour^{1, 2}, Tahir Alenezi^{1, 2}, Hong Wang², Danielle Graham², Billy M. Hargis², Xiaolun Sun^{2, 1}; ¹*Cell and Molecular Biology & Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*, ²*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*.

Necrotic enteritis (NE) is an acute enterotoxaemia enteritis in chickens. Although its pathogenesis remains largely unresolved, the severity was often associated with the toxin-releasing of *Clostridium perfringens*. In previous study, we found that immunized chickens with *C. perfringens* sporulation vaccines reduced chicken NE. The objective of this study was to identify the most effective doses and to investigate its mechanism. Vaccines labelled as CP1 and CP2 were prepared from sporulation supernatant proteins of two *C. perfringens* isolates. The vaccines were examined for CPE presence and epithelial and immune cellular toxicity. Cohorts of 12 birds per group were vaccinated at d 0 and 10 with different doses of CP1 and CP2 in adjuvant aluminum hydroxide. Birds were infected with 20,000 sporulated *E. maxima* M6 oocysts at d 16 and 10⁹ CFU/bird *C. perfringens* at d 20. Chicken body weight was measured at d 0, 16, 20 and 21. Birds were sacrificed at d 21 to collect blood, ileal tissue and content for analysis of antibody titer, histopathology, inflammation and colonization levels. Differences between treatments were analyzed using One-way ANOVA and nonparametric Mann–Whitney U test. Consistent with previous reports, *E. maxima* and *C. perfringens* induced clinical NE of severe intestinal inflammation showed as ileal villus shortening and necrosis, immune cell infiltration, and crypt hyperplasia. Notably, vaccines CP1 and CP2 strongly attenuated the intestinal inflammation. The NE also induced body weight gain (BWG) loss during d 20-21 (NE phase), while vaccinated birds resisted the BWG loss. Among these vaccine groups, birds vaccinated with CP1-1 achieved the highest daily BWG during NE phase compared to NE birds (25 vs -3g/bird/day). ELISA assay showed that antibody titer in CP1-1 was increased by 49 and 69%, respectively, compared to those in NC and NE birds. Real time PCR results showed that *C. perfringens* in ileum luminal and tissue of CP1-1 birds was reduced by 5 and 3 logs, respectively, compared to those in NE birds. In conclusion, vaccinating chickens with *C. perfringens* sporulation vaccine effectively reduces chicken NE, possibly through inducing protective antibody and reducing *C. perfringens* colonization and invasion.

Key Words: Necrotic enteritis, *Clostridium perfringens*, sporulation, dose optimization, vaccine

48 Immunomodulatory effects of mixed lactobacilli oral inoculation on experimentally induced necrotic enteritis in broiler chickens. Bahram Shojadoost*, Mohammadali Alizadeh, Nitish Boodhoo, Jake Astill, Janan Shoja Doost, Shayan Sharif; *Pathobiology, The University of Guelph, Guelph, Ontario, Canada*.

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Objectives: To evaluate the immunomodulatory effects of orally inoculated mixed lactobacilli strains on experimental necrotic enteritis (NE) in broiler chickens. **Experimental design:** Ninety day old male broiler chickens were randomly divided into 6 groups. Four groups orally received 10⁷ or 10⁸ CFU of a mixture of 5 lactobacilli isolates (*L. acidophilus*, *L. reuteri*, *L. crispatus* and two strains of *L. johnsonii*) at 1, 8, 15 and 20 days of age. Groups 5 and 6 as controls, did not receive lactobacilli. All groups challenged with 10⁸ of a pathogenic CP strain twice a day for 3 days starting at day 21. Before and after challenge, intestinal segments were collected to assess the expression of cytokines of the immune system by Realtime PCR. Meanwhile, samples collected from cecal tonsils were processed for flowcytometry. **Materials and Methods: RNA extraction and Realtime PCR:** Duodenum and jejunum segments were collected for RNA extraction, cDNA synthesis and Realtime PCR to evaluate the relative gene expression of interferon (IFN)- γ , interleukin (IL)-1 β , IL-2, IL-12, IL-13, IL-17, IL-18, Toll-like receptor (TLR)2 to β -actin. **Tissue processing and Flowcytometry:** Cecal tonsil cells were isolated, overlaid on Histopaque and the interface were collected and counted by haemocytometer method. The cells were then stained with antibodies to measure the frequency and total number of surface markers of CD3-CD8+, CD3+CD8+, CD3+CD4+, BU1+IgM+, BU1+IgY+ and BU1+IgA+ cells, using the FACS machine. **Statistical Analysis:** All cytokine gene expression data were statistically analyzed using GraphPad Prism 9, by One Way ANOVA, followed by Tukey's test and non-parametric Kruskal-Wallis test followed by Dunn's test when appropriate. The FACS data were analysed by Wilcoxon and Mann Whitney non-parametric to test significance as appropriate. Results were considered statistically significant at $P \leq 0.05$. **Results:** When chickens received the higher dose of lactobacilli a significant decrease ($P < 0.05$) of IL-1 β , IL-12, IL-17 and TGF- β and an increase ($P < 0.05$) of IL-2, IL-13 and TLR2 was observed in the intestine. However, the expression of IFN- γ and IL-1 β increased significantly ($P < 0.05$) in the intestine of chickens received the lower lactobacilli dose. The absolute number of CD3-CD8+ and CD3+CD8+ cells as well as frequency of BU1+IgM+, BU1+IgY+ and BU1+IgA+ cells were increased ($P < 0.05$) in the cecal tonsil of the chickens received the higher dose of lactobacilli. **Conclusion:** Oral inoculation of the mixed lactobacilli modulates the immune responses to maintain the homeostasis of the intestinal immune system when chickens are challenged with CP.

Key Words: Necrotic enteritis, *Clostridium perfringens*, gut health, immune system, broiler chickens

49 Essential oil and yeast cell wall efficacy in a necrotic enteritis challenge study. Charles L. Hofacre², Melina Bonato^{*1}, Liliana L. Borges¹, Ricardo L. Barbalho³; ¹*R&D, ICC Brazil, Sao Paulo, Sao Paulo, Brazil*, ²*Southern Poultry Research Group, Inc., Watkinsville, Georgia, United States*, ³*Sales, ICC Brazil, Sao Paulo, Sao Paulo, Brazil*.

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Currently, the international poultry industry is experiencing an increase in Necrotic Enteritis (NE) because of banning the use of growth-promoting antibiotics by some countries and/or current consumer preferences for decreased antibiotic use in food production. The objective of this study was to determine the effectiveness of either a new Essential Oil combined or not with a Yeast Cell Wall to reduce the negative effects of NE on broiler chickens. For this, 420 one-day-old Ross x Ross male chicks were distributed in a CRB design, with 6 treatments and 7 blocks with 10 birds per cage (0.44 m²/bird). The treatments were: T1- Negative Control [NC] (no additive supplemented); T2- Positive Control [PC] (Maxiban®, 72 g/US ton); T3- Essential Oil [EO300] (based on carvacrol, 300 g/MT); T4 - Essential Oil [EO600] (600 g/MT); T5 - EO300 + Yeast Cell Wall [YCW] from *Saccharomyces cerevisiae* (ImmunoWall®, 0.5 kg/MT); T6 - EO600 + YCW (0.5 kg/MT). Birds received crumble feed until 28 days. The challenge model consisted of coccidia from the *Eimeria maxima* on day 14 gavaged to each bird and *Clostridium perfringens* (CP), gavaged on days 19 and 20 (with 1.0 mL of a 1.0 x 10⁸ CFU/mL). Because desired mortality was reached, birds were not gavaged on day 21. On day 22, one bird per pen was humanely euthanized, weighed, necropsied, and lesion scored, according to: 0 = Normal; 1 = Slight mucus covering small intestine; 2 = Necrotic small intestine mucosa; 3 = Sloughed and blood small intestine mucosa and contents. All birds were weighed at days 0, 14, 22, and 28; feed intake was monitored. The statistical analysis performed (STATISTIX) were general linear procedures using ANOVA with a comparison of means using least significant difference (*t*-test) (LSD [T]) at 0.05. The NE lesions were compared using Kruskal-Wallis one-way nonparametric analysis of variance at 0.05. The CP challenge was greater than expected, with 37% NE mortality in the NC groups. However, all the treatments significantly reduced NE mortality, being the PC with the greatest reduction (20%), followed by EO300+YCW (27.14%), EO300 (28.57%), EO600+YCW (28.57%), and EO600 (32.86%). As often occurs, when NE mortality is high, there is not a significant difference in NE lesion scores, as we found in this trial. At 28 days, the EO300+YCW had higher significant BWG (1.38kg) compared to NC (1.29kg) and PC (1.31kg). The EO300+YCW had an adjusted FCR very similar (1.48) to the PC (1.49) and significantly better than EO600+YCW (1.56). Overall, with very strong CP, all the non-antibiotic treatments were effective in lowering NE mortality. These treatments also supported the birds' intestines and allowed them to convert feed to body weight efficiently.

Key Words: broilers, gut lesions, mortality, *Eimeria maxima*, *Clostridium perfringens*

50 Effect of a whole yeast product supplementation on broiler performance and intestinal necrotic lesions during an experimental *Clostridium perfringens* infection. Jose Charal*¹, Michael Sims², Brian Glover¹, Milan Hruby¹; ¹ADM Animal Nutrition, Quincy, Illinois, United States, ²Virginia Diversified Research Corp., Poultry Sci. 100 (E-Suppl 1)

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A 42-d floor pen study was conducted to evaluate the effects of whole yeast (*Pichia guilliermondii*; WYPG) product (CitriStim®) supplementation on broilers performance, mortality, and lesions related to necrotic enteritis following an experimental *Clostridium perfringens* infection. Newly hatched Ross 708 broilers (1:1 sexed female to male) were allotted to 4 treatments and 12 replicates with 30 birds per pen. Birds were fed a 3-phase feeding program and the treatments consisted of 1) Non infected control (NIC) 2) Infected control (IC) 3) IC + WYPG low dosage (1.0 kg/MT until d 28 then 0.75 kg/MT) and 4) IC + WYPG high dosage (1.5 kg/MT until d 28 then 1.0 kg/MT). Used litter was added to each pen on day 4 (454 g), and birds were challenged with *C. perfringens* on d 17, 18, and 19. Birds were weighed on d 14, 28, and 42. On d 21, 3 birds per pen were randomly selected, euthanized and the intestines examined and scored for *C. perfringens* lesions. Lesion scores were based on a scale 0 to 4 with 0 as normal and 4 as the most severe lesions. Compared to NIC, challenged birds (IC) had lighter BW at d 42 and the supplementation with WYPG ameliorated the reduction on BW (P < 0.05). At d 42, adjusted feed conversion ratio (FCR) was higher for IC compared to NIC and WYPG supplemented birds had intermediate FCR (P < 0.05). Challenged birds (IC) had numerically higher overall mortality rate compared to NIC (7.22 vs 4.17%; P > 0.05). Lesion scores were higher in IC birds, and the supplementation of WYPG resulted in challenged birds with reduced necrotic intestinal lesions scores (P < 0.05). These results suggest that supplementation of whole yeast (WYPG) product during a *C. perfringens* challenge may reduce the impact of necrotic enteritis on broiler performance and on severity of intestinal necrotic lesions.

Key Words: *Clostridium perfringens*, whole yeast, mortality, adjusted FCR, necrotic enteritis

51 Development of antigen-capture ELISAs for the detection of necrotic enteritis B-like (NetB) toxin and collagen adhesin protein (CNA) in broiler chickens afflicted with necrotic enteritis. Doyun Goo*, Youngsub Lee, Hyoyoun Nam, Mingmin Lu, Charles Li, Hyun Lillehoj; *Animal Bioscience and Biotechnology Laboratory, USDA-ARS, Beltsville, Maryland, United States.*

Clostridium perfringens is a causative pathogen of poultry necrotic enteritis (NE). Among the many virulent toxins produced from *C. perfringens*, necrotic enteritis B-like (NetB) toxin is known as the main virulent factor of NE. Collagen adhesin protein (CNA) is a *C. perfringens* protein involved in collagen-binding activity of bacteria and plays a key role in the NE pathogenesis since CNA has adhesive properties to attach to host extracellular matrix such as collagen. Thus, early detection of CNA and NetB antigens in biological samples from NE-afflicted chickens can be a reliable indicator of NE status. Newly developed mouse monoclonal antibodies (mAbs) detecting CNA and NetB proteins of *C. perfringens* were used to develop sensitive

antigen-capture ELISA assays for early detection of NE in serum, gut content and fecal samples from commercial broiler chickens undergoing NE. Out of more than 100 hybridoma clones, best antigen capture-detection pairs for CNA and NetB ELISAs were selected and used to develop sensitive detection assays. For collecting biological samples, all broiler chickens were infected with *Eimeria maxima* 41A followed by NetB-positive Del-1 *C. perfringens* according to our NE model. All samples including serum, gut content, and fecal samples were obtained at 48 h post *C. perfringens* infection. Correlation coefficient and standard curves were calculated and analyzed by GraphPad Prism. Results indicate that CNA or NetB toxin can be detected in the gut contents and fecal samples but not in serum of NE-infected chickens. The average levels of CNA and NetB toxins measured were 833 ng/ml and 2,275 ng/ml, respectively, in all *C. perfringens* infected gut contents. In addition, the average levels of CNA and NetB toxins in *C. perfringens* infected fecal samples were 850 ng/ml and 1,182 ng/ml, respectively. Pearson's correlation coefficient excluding non-detected samples showed a positive linear relationship and a strong association ($r^2 = +0.817$) between the amounts of CNA and NetB toxin in the gut digesta and fecal samples. In conclusion, CNA- and NetB-specific antigen capture ELISAs will be valuable monitoring tools for early detection of NE outbreak in large poultry farms.

Key Words: broiler, *Clostridium perfringens*, collagen adhesin protein, ELISA, NetB toxin

52 Early life exposure to cold stress causes distinct changes in broiler chicken gut neurochemistry and microbiome that persist into later life. Joshua M. Lyte^{*1}, Julia Eckenberger², James Keane³, Kelsy Robinson¹, Sandip Shrestha⁴, Annie M. Donoghue¹, Valentina Caputi⁴, Karrie Daniels⁵, Mark Lyte⁵; ¹USDA-ARS Poultry Production and Product Safety Research Unit, Fayetteville, Arkansas, United States, ²Department of Microbiology, University College Cork, Cork, Ireland, ³Department of Computer Science, Cork Institute of Technology, Cork, Ireland, ⁴Department of Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, ⁵Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, Iowa, United States.

Objective: Early life stress can cause persistent neuroendocrine adaptations that increase susceptibility to infection in later life. Stress-related neurochemicals produced in the gut play a critical role in enteric colonization and host-microbe bi-directional communication. Young chicks are particularly vulnerable to cold stress, yet it is unknown whether cold exposure during early life may shape neuroendocrine development of physiological sites where foodborne pathogen colonization primarily occurs, such as the gut. We therefore determined if cold stress experienced during the first week post-hatch would cause immediate and prolonged alterations in chicken enteric neurochemical concentrations and the

microbiome. **Experimental design:** Broiler chicks at hatch were randomly divided into control or cold stress groups. Chicks from both groups (n=10/group) were euthanized at 3 days old to assess baseline differences. Chicks were subjected to cold stress at days 4, 5, and 6 post-hatch. Cold stress consisted of an ambient room temperature of 20°C for 6 hour/day. Birds (n=10/group) were euthanized at 7, 9, and 21 days old. **Materials and Methods:** Quantification of neurochemicals in ileum and cecum tissues, as well as ileal and cecal contents were determined using ultra-high performance liquid chromatography with electrochemical detection. qPCR of neurochemical-related genes was assessed in ileal and cecal tissues. 16S analysis of the cecal microbiome was performed. **Statement of statistical analyses:** Data were analyzed by two-way ANOVA with Dunnett's or Sidak's posthoc test, where appropriate. **Results:** Norepinephrine and serotonin were increased (p<0.05) in the cecal content and tissue, respectively, of cold stressed but not control birds. The main metabolite of serotonin, 5-hydroxyindoleacetic acid, was increased in cold stressed cecal tissue. Ileal tissue and content norepinephrine concentrations diverged (p<0.05) between cold stressed and control birds. Cold stress caused (p<0.05) the downregulation of expression of serotonin and transporter genes in the ileum, and an increase (p<0.05) in cecal serotonin receptor expression. Cecal microbiome diversity was decreased in cold-stressed chickens at days 7 and 9 of age but recovered at day 21 while differences in microbial composition remained throughout the tested period. **Conclusions:** Cold stress caused immediate and prolonged changes in the broiler cecal microbiome, as well as gut neurochemical concentrations and related gene expression in both the ileum and cecum. Further studies are required to determine the implications of these findings in mediating stress-induced susceptibility to enteric colonization by foodborne pathogens.

Key Words: stress, gut, microbiome, neurochemical, norepinephrine

53 Withdrawn.

54 Candidate genes for A and E blood group systems in the chicken. Robert L. Taylor^{*1}, Wioleta Drobik-Czwaro², Anna Wolc^{3, 4}, Janet Fulton⁴; ¹West Virginia University, Morgantown, West Virginia, United States, ²Department of Animal Genetics and Breeding, Warsaw University of Life Sciences, Warsaw, Poland, ³Department of Animal Science, Iowa State University, Ames, Iowa, United States, ⁴Hy-Line International, Dallas Center, Iowa, United States.

The A and E alloantigens are two of 13 blood group systems identified on chicken red blood cells. Variants are detected with blood group-specific alloantisera. The gene products and chromosomal location of the B blood group, (major histocompatibility complex, MHC), have been identified, but equivalent information is not known for the other blood groups. Blood group systems A and E have seven and

eleven known alleles respectively. The two systems are genetically linked at 0.5cM, estimated by classical recombination studies. The objective of this study was to determine the chromosomal location of the genes encoding the A and E system alloantigens. Multiple genetic resources were available, including genomic sequences of inbred or selected lines with known A, E or both system alleles, NIU DNA bank samples for which one or both systems had been serotyped, plus DNA from Hy-Line International lines with A system allele information. Furthermore, an A system-specific monoclonal antibody (ISU-cA) was available to test additional samples. Sequence information plus genome wide association analyses of White Leghorn and Rhode Island Red samples with differing ISU-cA antibody reactivity, revealed the same single strong peak on chromosome 26 at 2.6-2.7Mb (Galgal7) for both breeds. Regression analysis of SNP from 600K Chicken Axiom arrays from individuals of known A alleles confirmed a SNP relationship with phenotype in the same region. Three criteria were used to identify candidate genes; genes must be expressed on the cell surface, SNP must be in exons, and produce predicted change in the encoded protein. One gene in this region fit all criteria; C4BPM (complement 4 binding protein, membrane bound), a member of the Regulators of Complement Activation gene cluster. SNP genotypes and subsequent haplotypes of C4BPM determined in independent sets of samples confirmed the association between variation within C4BPM and A system alleles. For chicken chromosome 26, 0.5cM is estimated to be equivalent to 33,750bp. While the A and E systems are reported to be within 0.5cM of each other, a more inclusive region from 4.5cM downstream to 6.8cM upstream of C4BPA was examined in detail for possible E system candidates. The gene LOC101748581, located 89,000 bp (2.64cM) from C4BPM, showed the best fit between sequence data SNP variation from samples with distinct E system alleles and the E system phenotypes. Limited sample numbers precluded unequivocal identification of the E system candidate gene, so another unknown candidate gene may lie within this sparsely annotated genomic region.

Key Words: alloantigen, blood group, C4BPA, allele

55 Characterization of the effects of *Salmonella Typhimurium* on circulatory exosomal miRNA profiles in chicks. Dan Zhao¹, Wenli Li⁴, Morgan Farnell¹, Michael Kogut², Kenneth J. Genovese², Chuan-Yu Hsu³, Haiqi He², Adela Oliva Chavez⁵, Yuhua Z. Farnell^{*1}; ¹Poultry Science, Texas A&M AgriLife Research/Texas A&M University, College Station, Texas, United States, ²USDA-ARS, College Station, Texas, United States, ³IGBB/Mississippi State University, Mississippi State, Mississippi, United States, ⁴S Dairy Forage Research Center, Madison, Wisconsin, United States, ⁵Department of Entomology, Texas A&M University, College Station, Texas, United States.

Molecular signatures present in exosomes, especially microRNAs (miRNA), play essential roles in intercellular

communication and have been implicated as novel mediators in host-pathogen interactions. However, the mechanism of circulatory exosomes between chicken and *Salmonella Typhimurium* (ST) remains to be elucidated. To understand the role of miRNA in regulating genes involved in the host response to ST infection, next-generation sequencing was applied to investigate differentially expressed (DE) exosomal miRNAs in serum of broilers after ST challenge. In this study, day-old broiler chicks were randomly divided into two treatment groups, orally inoculated with 0.5 mL of 10⁹ CFU (ST challenged group; n = 183) or PBS (Control group; n = 190). Blood samples were collected 24 h post-challenge. Exosomes were extracted from serum, and their morphology and concentration were characterized by transmission electron microscopy and Nanosight tracking analysis. The concentration, mean size, and mode of exosomes were not significantly different in the ST group compared to the Control group. However, miRNA profiles in serum exosomes of the ST group were significantly different from those in the Control group. Seventeen miRNAs upregulated and 12 miRNAs downregulated in the ST- infected birds compared to the Control birds (P < 0.05). The top 6 upregulated exosomal miRNAs in ST birds belonged to gga-miR-34b/c and gga-miR-449d/a/b/c families, the largest set of significant clusters of miRNAs, sharing the common two seeding sequences GGCAGUGU and GUUAGCUG. These miRNAs were implicated to target genes that are involved in modulating humoral, innate, and adaptive immune responses, cytokine-mediated signaling pathway, B cell proliferation and differentiation, activating nature killer cells and T cells, positive regulation of peptidyl-serine phosphorylation of STAT protein, and Notch signaling pathway (P<0.05). Overall, the ST infection leads to alterations of the miRNA profiles in circulating exosomes, suggesting that exosomal miRNA act as important modulators for host-pathogen interaction in broilers.

Key Words: MiRNA, exosomes, *Salmonella typhimurium*, broiler, next-generation sequencing

56 Efficacy of protected benzoic acid on gut histology of broilers with or without an antibiotic as growth promoter under commercial conditions. Raquel B. Araujo², Flavio A. Longo², Ricardo A. Ivanovski², Fabricio B. Duarte², Bruna L. Belote^{*1,3}, Huendy F. Moreira⁴, Vitor C. Fonseca⁴; ¹Department of Veterinary Science, Federal University of Parana, Curitiba, Brazil, ²Novus do Brasil Comércio e Importação Ltda, Indaiatuba, São Paulo, Brazil, ³ISI Institute, Curitiba, Brazil, ⁴Granja Brasília Ltda, Pará de Minas, Minas Gerais, Brazil.

It is recognized that the benefits of eubiotic feed additives are more consistently demonstrated under challenge. Although challenges are applied in research facilities, the effects of eubiotics under field conditions should be evaluated. The aim of these studies was to determine the effect of protected benzoic acid (AVIMATRIX® Novus

International, Inc., PBA, 500g/MT) product in feed on gut histology of broilers under commercial conditions. Two trials were conducted in commercial facilities in Brazil, in conventional barns for 30,000 birds with similar historical performance and challenges. In trial 1, 4 barns received standard diets (including Halquinol as growth promoter, Control) while 5 were supplemented with PBA (on top). In trial 2, 3 barns were fed with Control diets and 5 received PBA in replacement of Halquinol (30 g/MT). On day 28, in both trials, 3 broilers per barn with the average body weight $\pm 2\%$ were euthanized to collect ileum samples for histology analysis (20 villi per bird) applying the I See Inside (ISI) method. This methodology considers the intensity of histological lesions and the impact of each of the 8 parameters to organ function. A score is obtained for each parameter and the sum of all scores is analyzed as well. Data were submitted to Shapiro-Wilk normality test and Kruskal-Wallis test at 5% using 27 samples in trial 1 and 24 in trial 2. In trial 1, the group supplemented with PBA in the diet presented better results than Control, showing lower score of lamina propria thickness (1.54 vs 1.79, $P < 0.01$), epithelial thickness (0.65 vs 0.81, $P < 0.01$), proliferation of enterocytes (0.66 vs 0.86, $P < 0.001$), inflammatory cell infiltration on epithelium (0.50 vs 0.62, $P < 0.01$), inflammatory cell infiltration in lamina propria (0.73 vs 0.92, $P < 0.05$) and in the sum of all scores (5.10 vs 5.89, $P < 0.05$). In trial 2, broilers supplemented with PBA showed again better results than Control group, presenting lower score of lamina propria thickness (1.87 vs 2.08, $P < 0.01$) and inflammatory cell infiltration in lamina propria (0.88 vs 1.20, $P < 0.01$). In addition, PBA tended to reduce epithelial thickness score (0.65 vs 0.74, $P < 0.10$) and reduced the sum of all scores (5.52 vs 6.17, $P < 0.05$). There were no differences between treatments for goblet cells, congestion and presence of oocysts in both trials ($P > 0.05$). In conclusion, broilers under commercial challenges and supplemented with PBA (in diets with or without Halquinol as growth promoter) improved gut morphology by reducing intestinal inflammation and epithelial cell turnover, which may positively reflect in performance due to better absorption capacity and improved gut barrier.

Key Words: I See Inside, Avimatrix, eubiotic, small intestine, gut health

57 Effects of *in ovo* co-administration of vitamins (A and D) and probiotic lactobacilli on cell- and antibody-mediated immune responses in newly hatched chickens.

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Hypothesis and Objectives: Newly hatched chicks experience stressful conditions during hatchery procedures

that might lead to compromised immune function and enhance the potential risk of infectious diseases in neonatal chicks. There is some evidence that probiotic lactobacilli in addition to essential vitamins such as vitamin A and D have immunomodulatory properties that might strengthen the immune system of neonatal chickens against post-hatch infection. The present study was conducted to determine the effects of *in ovo* inoculation of retinoic acid (RA), 25-Hydroxyvitamin D₃ (VitD), either alone, or in combination with probiotic lactobacilli on cytokine gene expression, spleen cell subsets, and antibody-mediated immune responses in chickens. **Materials and Methods:** At embryonic day 18, three hundred and sixty eggs were randomly assigned to 9 experimental groups. Lactobacilli (10^7 CFU/egg), RA (90 $\mu\text{mol/egg}$), and VitD (0.6 $\mu\text{g/egg}$) were administered *in ovo*. In addition, some groups received lactobacilli with either RA or VitD. Untreated control remained uninjected. On day 5 and 10 post-hatch, 6 birds per treatment were euthanized and spleen, and the bursa of Fabricius were collected for gene expression and cell composition analysis. To assess antibody-mediated immune response, birds were immunized on days 14 and 21 post-hatch with sheep red blood cells (SRBC) and keyhole limpet hemocyanin (KLH), and sera were collected (12 birds per treatment) on days 7, 14, and 21 post-primary immunization for serological analysis. Data were analyzed using the generalized linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC), and differences among treatments means were determined using Tukey's multiple comparison test. **Results:** Lactobacilli treatment increased the number of monocyte/macrophages (KUL01⁺) and CD3⁺CD4⁺ T cells in the spleen, and enhanced serum anti-KLH IgM and IgY on day 14 post-primary immunization ($P < 0.05$). However, expression of the cytokines was not affected with either lactobacilli or VitD treatment ($P > 0.05$). *In ovo* inoculation of RA significantly increased serum IgY and IgM titer to KLH and enhanced expression of interferon (*IFN*)- α , *IFN*- β , interleukin (IL)-1 β , IL-6, IL-8, IL-12, IL-13, and transforming growth factor- β in the bursa of Fabricius ($P < 0.05$). The percentage of monocyte/macrophages (KUL01⁺), CD3⁺CD8⁺ NK cells, and CD3⁺CD8⁺ T cells was elevated in the spleen as well ($P < 0.05$). **Conclusions:** These findings indicate that pre-hatch administration of RA has potent immunoadjuvant activities that might protect newly-hatched chicks against early infection.

Key Words: In ovo, Retinoic acid, Chickens, Cytokine, Antibodies

58 Comparison of chick quality, health, and inflammation from two hatchery environments.

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Hatchery contamination can result in microbial exposure that may negatively impact chick quality and early health. Chicks exposed to pathobionts within hatcheries may ingest

or inhale these pathogens, resulting in modified colonization of intestines and respiratory tract, with the potential to influence early growth development, inflammation, and overall health. Six experiments were completed to compare chick quality, health, and inflammation between two hatcheries (H1 and H2). On embryonic d0, 45 eggs from the same breeder flock were set at each hatchery. On day of hatch (DOH), length, abdominal height, navel and leg abnormalities, and self-righting were measured (36 chicks/hatchery), yolk sacs were weighed, and crop/cloaca swabs were cultured (12 chicks/hatchery). On d7, mid-ileum and ceca were cultured (12 chicks/hatchery). On DOH and d7, body weight (BW) and intestinal weight was measured, lung/air sac swabs and liver were cultured, liver and air sacs were scored for health, and blood was collected for serum alpha-1-acid glycoprotein (A1GP) concentrations in Exp 1 and 2 (12 chicks/hatchery). Data was analyzed using t-test or χ^2 , significance $p < 0.05$. On DOH, BW, length, yolk free BW, and intestinal weight were greater for H2 chicks ($p < 0.05$). Liver bacterial recovery was decreased in H2 compared to H1 on DOH ($p < 0.05$) and there were fewer navel, leg, and righting abnormalities noted in H2 ($p < 0.05$). Decreased instances of lactase positive Enterobacteriaceae were noted in H2 in both crop/cloaca and lung/air sac swabs on DOH ($p < 0.05$), as well as fewer occurrences of alpha and beta hemolysis in crop cloaca swabs, and alpha and gamma hemolysis in lung/air sac swabs on DOH ($p < 0.05$). By d7, most differences were abated other than an increase in alpha hemolytic bacteria in lung/air sac swabs of H2. Though A1GP was not different between H1 and H2 on DOH or d7, and there was no time by treatment interaction ($p > 0.05$), A1GP concentrations of H2 nearly doubled while H1 increased by 12%, suggestive of a change in inflammatory status over the first week, and a potential change in responsiveness to inflammation. Differences observed on DOH for health metrics and microbial colonization showed chicks from H2 to have improved health and starting quality based on factors measured. While not sustained through d7, differences in DOH microbial recovery, such as greater incidence of lactase positive Enterobacteriaceae and beta hemolysis in crop/cloaca samples, may have shifted microbial development with the ability to influence immune response. These experiments were able to elucidate the importance of hatchery environment on early chick quality, microbial colonization, generalized inflammation, and overall chick health.

Key Words: chick quality, hatchery environment, inflammation, microbial colonization

59 *Histomonas meleagridis* and cholesterol depletion in turkeys. Vijay Durairaj*, Deborrah Higuchi, Ryan V. Veen; *Huvepharma Inc, Lincoln, Nebraska, United States.*

Histomoniasis is a deadly disease in turkeys caused by the protozoan, *Histomonas meleagridis*. Histomoniasis causes severe lesions in ceca and the liver. The diagnosis of histomoniasis is based on the pathology and isolation of the

organism. By the time of disease confirmation, most of the affected turkeys in the flock are usually dead. Thus, an early diagnosis of histomoniasis would be helpful. The main hypothesis of this research is to evaluate if the serum clinical chemistry provides any valuable information towards the early diagnosis of histomoniasis. In study 1, day-of-hatch poult (n=29) were assigned into three groups - Group 1 (n=9), Group 2 (n=10), and Group 3 (n=10). On study day 27, Groups 2 and 3 were challenged with *H. meleagridis* - HMA at 10^3 organisms/dose by intra-cloacal inoculation and cloacal-drop method, respectively. Group 1 served as a negative control. Five poult from each group were bled and necropsied on study day 36 (9 days post-challenge). All the poult in Group 2 (n=5) and 3/5 poult in Group 3 experienced mortality prior to the final necropsy on study day 41 (14 days post-challenge), preventing blood sample collection from the dead birds. Cecal and liver lesion scores (0-4 scale) were recorded for each poult. Group 2 and 3 had 100% histomoniasis incidences. Group 1 poult had an average cholesterol level of 136 ± 14.34 mg/dL, while Groups 2 and 3 had average cholesterol levels of 57 ± 12.65 mg/dL and 75.6 ± 13.92 mg/dL, respectively. In study 2, day-of-hatch poult (n=34) were assigned into three Groups - Group 1 (n=4), Group 2 (n=15) and Group 3 (n=15). On study day 15, Groups 2 and 3 were challenged with *H. meleagridis* - HMA at 10^4 and 10^5 organisms/dose, respectively, by intra-cloacal inoculation. Group 1 served as a negative control. Poult were bled and necropsied on study day 24 (9 days post-challenge). Cecal and liver lesion scores (0-4 scale) were recorded for all poult. Group 2 and 3 had 93% and 100% histomoniasis incidences, respectively. Group 1 poult had an average cholesterol level of 195.25 ± 22.08 mg/dL, while Groups 2 and 3 had an average cholesterol levels of 96.27 ± 33.96 mg/dL and 61.87 ± 17.40 mg/dL respectively. In both studies, poult infected with *H. meleagridis* - HMA had significantly reduced cholesterol levels based on one-way ANOVA analysis. In summary, *H. meleagridis* challenge resulted in reduced cholesterol levels in the poult, indicating that the serum cholesterol profile could provide more insights in the early diagnosis of histomoniasis prior to high mortality. Evaluation of the serum cholesterol profile, along with existing diagnostic tools, could help in the early detection of histomoniasis.

Key Words: *Histomonas meleagridis*, histomoniasis, turkeys, serum clinical chemistry, cholesterol

60 Impact of laying hens alternative housing systems on the incidence of intestinal and external parasites.

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Due to consumers demand, alternative housing systems are rapidly expanding in Quebec and Canada. Egg farmers may choose to invest in enriched colony housing system or

aviaries, but with the trend for free roaming hens, some 'old' diseases are re-emerging. Coccidiosis, caused by protozoan *Eimeria* species, has an impact on intestinal integrity, and might allow for the development of necrotic enteritis. Nematodes, including Capillariidae, and Ascarididae, can also affect the health of laying hens as well as egg quality, while red mites (*Dermanyssus gallinae*), can cause serious production losses. Our hypothesis is that the incidence of pathogens and associated diseases is higher in aviaries than in enriched colony housing systems. Objectives were to compare *Eimeria* excretion curves and the incidence of the intestinal and external parasites between the two egg laying housing systems between 19 to 65 weeks of age. **Experimental design:** The study was carried out from fall 2017 to spring 2021. Twenty-four egg barns (12 aviaries and 12 enriched colony housing systems) were selected and monitored between placement (19 weeks of age) and 65 weeks of age. **Materials and methods:** Egg farmers collected weekly composite fecal samples for the duration of the study. Parasitology analysis was performed on samples from weeks 20, 24, 26, 37, 43, 57, 60, and 63. These weeks were selected because of the lesser number of missing samples. For intestinal parasites (*Eimeria* spp, Ascaridia and Capillaria) McMaster and Wisconsin sugar floatation tests were used for quantification, and identification of *Eimeria* spp. oocysts, Ascaridia and Capillaria was done. Red mite traps were placed throughout twenty egg barns on week 55, and collected for analysis 48 hours later. Statistical analyses were carried out with R statistical software. Generalized mixed multivariate models were used. **Results:** There was a statistically significant effect of housing type on the presence of *Eimeria* spp., with a higher number of aviaries showing larger numbers of oocysts. Age had a significant and negative effect on the presence of *Eimeria* spp, with younger hens showing larger number of oocysts. There was no season effect. No Ascaridia nor Capillaria eggs were found in tested egg barns whether aviaries or enriched colony-housing systems. No trace of red mites was detected in both systems. **Conclusions:** Given the larger number of *Eimeria* spp. oocysts detected in the fecal samples of hens housed in aviaries, it would be important to properly vaccinate replacement pullets. Surprisingly, there were a few fecal samples from enriched colony housing systems showing *Eimeria* spp. oocysts. These cases should be further investigated and vaccination considered.

Key Words: alternative housing systems, intestinal parasites, *Eimeria*, nematodes, red mites

61 Impact of laying hens alternative housing systems on the incidence of *Clostridium perfringens* and NetB toxin. Maude Lanoie, Rachid Chebta, Lila Maduro, Martine Boulianne*; *Clinical Sciences, Faculté de Médecine Vétérinaire, Université de Montréal, St. Hyacinthe, Quebec, Canada.*

In Canada, the egg industry will ban all conventional cages by 2036. Egg farmers have to choose to invest in enriched

colony housing system or aviaries, but with the trend for free roaming hens, greater contact of the birds with litter, some 'old' diseases are re-emerging. *Clostridium perfringens* (Cp) is a Gram positive bacteria found in the intestines of chickens. *Clostridium perfringens* strains have been shown to vary in virulence with those harbouring the NetB toxin being associated with necrotic enteritis. Episodes of necrotic enteritis and mortality have been reported in hens housed in aviaries. Our hypothesis is that the incidence of *Clostridium perfringens* and the NetB toxin is higher in aviaries than in enriched colony housing systems. Objectives were to compare these parameters for two egg laying alternative housing systems between 20 to 65 weeks of age. The study was carried out from the fall 2017 to spring 2021. Twenty-four egg barns (12 aviaries and 12 enriched colony housing systems) were selected and monitored between placement (19 weeks of age) and 65 weeks of age. Egg farmers collected weekly composite droppings samples for the duration of the study. Bacteriological isolation of *Clostridium perfringens* was performed on samples from weeks 20, 24, 26, 37, 43, 57, 60, and 63. DNA was extracted with Chelex and then amplified by multiplex PCR to determine the toxin type. A mixed generalized linear model with housing type, age and season as a fixed factor was used to analyze the incidence of *Clostridium perfringens* as confirmed by the presence or the *cpa* gene as well as NetB toxin. There was no significant difference in the incidence of *Clostridium perfringens* between the two housing systems. The *cpa* gene, hence *Clostridium perfringens*, was detected in 85% of the samples from enriched colony housing systems ($n = 207$) and in 98% from those of aviaries ($n = 216$). NetB toxin was significantly more present in aviaries than enriched cages (CI = 0.54 - 8.49). Presence of NetB toxin also decreased with age (CI = -0.04 - -0.04). Finally, *Clostridium perfringens* was less present in winter (CI = -1.94 - -0.19) while the NetB toxin was less present in winter (CI = -4.31 - -0.99) and more present in summer (CI = 0.03 - 2.70). Episodes of necrotic enteritis were observed during the study in a few aviaries. This was corroborated by a large number of NetB positive *Clostridium perfringens* strains observed in aviaries. These cases should be further investigated to verify for any association with coccidiosis.

Key Words: *Clostridium perfringens*, *cpa* gene, netB toxin, alternative housing system, laying hens

62 No presentation materials submitted.

63 Investigating intestinal integrity, endogenous losses of amino acids, and nutrient digestibility of broiler chickens in response to graded severity of *E. maxima* infection. Po-Yun Teng^{GS*}, Janghan Choi, Sudhir Yadav, Yuguo H. Tompkins, Woo K. Kim; *Department of Poultry Science, University of Georgia, Athens, Georgia, United States.*

The study was conducted in two parts where the first part investigated the impacts of graded infection severity of

Eimeria maxima on intestinal health and amino acid digestibility, and the second part determined endogenous losses of amino acids in broiler chickens. In the first part of the study, there were four different levels of *E. maxima*-challenged treatments: a non-challenged control group, a low challenge dose (12,500 oocysts), a medium challenge dose (25,000 oocysts), and a high challenge dose (50,000 oocysts). There were eight replicate cages per treatment, with 12 birds per cage. Chickens in the challenged groups were orally challenged with sporulated oocysts per os on d 14. Intestinal morphology, amino acid digestibility, and gene expression of nutrient transporters and tight junction proteins were determined at 6 days post-infection. The orthogonal polynomial contrast within PROC GLM in the SAS software was used to perform the linear and quadratic statistical analyses, and the significance level was set at $P < 0.05$. The results showed a linear reduction in body weight gain, jejunal villus height, and intestinal integrity with the graded increase in doses of *E. maxima* ($P < 0.01$). Moreover, linear regulation of tight junction proteins expression was a consequence of increasing *Eimeria* challenge ($P < 0.01$). Furthermore, the expression of nutrient transporters and apparent ileal digestibility of amino acids were both down-regulated ($P < 0.01$) with increasing *E. maxima* inoculation dose. The second part of the study is a parallel experiment of the first one, except that the chickens were fed a nitrogen-free diet (NFD) from 0 to 6 days post-infection to determine endogenous losses during coccidiosis. The results indicated that endogenous losses of amino acids, except tryptophan, were linearly increased in response to the graded *E. maxima* challenge ($P < 0.05$). However, NFD reduced intestinal lesion scores, which might underestimate the true endogenous losses and standard ileal digestibility of *Eimeria*-infected chickens. In conclusion, increasing *E. maxima* infection severity linearly reduced body weight gain, intestinal integrity, and ileal digestibility of amino acids, and increased endogenous losses in broiler chickens. This study validates that *E. maxima* poses detrimental impacts on gut health and nutrient digestibility, leading to retardation in growth in a dose-dependent manner.

Key Words: coccidiosis, *Eimeria maxima*, endogenous loss, digestibility, intestinal integrity

64 Changes in the mRNA abundance of the stem cell marker gene *Olfm4* following a mild *Eimeria acervulina* and *E. maxima* challenge in broiler chickens.

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Infection with the protozoan parasite *Eimeria* causes the economically devastating disease coccidiosis, which is characterized by gross tissue damage and inflammation resulting in blunted villi. Previous studies have shown that *Eimeria* infection induces crypt lengthening, which is likely

to compensate for damaged villi, but no study has evaluated stem cell expression during this time. Two studies were conducted with 21-day old broiler chickens challenged with a mild dose of oocysts (100,000 *E. acervulina* or 1,000 *E. maxima*) or sham infected with sterile water. Duodenal or jejunal samples were collected from the *E. acervulina* or *E. maxima* groups, respectively, and their respective controls on the day of infection (0), 3, 5, 7, 10, and 14 days post-infection (dpi). The objective of these studies was to characterize the change in intestinal morphology during a mild *Eimeria* infection. Villus height (VH) and crypt depth (CD) were measured on bright field microscopy images following in situ hybridization for *Olfactomedin 4* (*Olfm4*) mRNA using the RNAscope methodology. The mRNA abundance of *Olfm4* was quantified by qRT-PCR and the results were analyzed by infection status using a t-test. Intestinal morphology data were analyzed using the non-parametric Welch's unequal variances test. In the *E. maxima* study mRNA abundance of *Olfm4* was upregulated at 5 dpi ($P = 0.017$); while it was unchanged throughout the *E. acervulina* study. In the *E. acervulina* study infected birds had shorter VH than uninfected birds at 5, 7, and 10 dpi ($P < 0.001$). In the *E. maxima* study infected birds had shorter VH than uninfected birds at 5 and 7 dpi; however, at 14 dpi infected birds had greater VH than the uninfected birds ($P < 0.01$). In contrast, in both studies infected birds had consistently increased CD. In the *E. acervulina* study, CD increased starting at 3 dpi and continued through 14 dpi ($P < 0.001$); while in the *E. maxima* study CD only significantly increased at 5 dpi ($P = 0.02$), with a non-significant increase in CD from 3 to 14 dpi. Interestingly, there was not a comparable increase in *Olfm4* mRNA abundance consistent with the increase in *Olfm4* expressing stem cells in the crypt as determined by in situ hybridization in the *E. acervulina* study. Further work is needed to clarify the relationship between the *Olfm4* mRNA quantification and in situ hybridization results. The expanded area of the *Olfm4*-expressing cells in the crypt is similar to the previously observed increase in the CD. This may provide an increase in stem cell populations to ensure an adequate supply of progenitor cells to replace damaged villus cells.

Key Words: coccidiosis, *Olfm4*, crypt depth, *Eimeria acervulina*, *Eimeria maxima*

65 Effects of *Eimeria* challenge on performance, body composition, and intestinal health of Hy-Line W-36 pullets. Milan K. Sharma*^{GS}, Po-Yun Teng, Yuguo H. Tompkins, Woo K. Kim; *Department of Poultry Science, University of Georgia, Athens, Georgia, United States*.

The current experiment was aimed to investigate the effects of *Eimeria* challenge on performance, body composition, and intestinal health of Hy-Line W-36 pullets. A total of 540, 16-d 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* oocysts per mL was prepared and

challenged to one group as a high-dose treatment. The 2-fold serial dilution was done to prepare the medium-high (25,000 *E. maxima*, 25,000 *E. tenella* and 125,000 *E. acervulina*), medium-low (12,500 *E. maxima*, 12,500 *E. tenella* and 62,500 *E. acervulina*), and low (6,250 *E. maxima*, 6,250 *E. tenella* and 31,250 *E. acervulina*) dose treatments and inoculate the three remaining groups, respectively. Growth performance, daily feed intake (FI) and mortality were calculated from 0-14 d post-infection (DPI). Dual Energy X-ray Densitometry was used to measure the body composition of pullets on 6 DPI. Gastrointestinal permeability (GP) was measured on 3, 5, 6, 7 and 9 DPI using fluorescein isothiocyanate dextran, whereas intestinal lesion scoring was done on 6 DPI. Data analyses for growth performance, body composition and GP were performed using one-way ANOVA. In contrast, the Kruskal-Wallis test was used for lesion score and split-plot in time for FI. A significant level was set at $P \leq 0.05$, and means were separated using Tukey HSD. The results indicated a significant linear reduction in average body weight (BW; $P=0.016$) and body weight gain (BWG; $P=0.011$). The body composition of birds (tissue weight, muscle weight, bone mineral density, and bone mineral content) linearly decreased with the increase in oocyst concentration ($P < 0.0001$). An interaction between treatment and DPI was observed for FI ($P < 0.0001$). Feed intake significantly dropped from 4 DPI and did not recover until 12 DPI in the challenged groups. The lowest FI for each of the challenged groups was observed on 5 DPI. Gastrointestinal permeability increased linearly, peaking at 5 DPI and recovered back to normal by 9 DPI on challenged groups. The intestinal lesion scores were higher in the challenged groups than the control ($P < 0.05$); however, no significant differences were observed among the challenged groups. The highest mortality was observed in pullets challenged with the high dose followed by the med-high dose ($P < 0.0001$) on 5 DPI. It can be concluded that the mixed *Eimeria* challenge linearly reduced the BW, BWG, and altered body composition of pullets with an increase in oocyst concentration. A severe effect of *Eimeria* on intestinal health might be observed on 5 DPI as suggested by GP and mortality results.

Key Words: pullets, *Eimeria*, coccidiosis, gastrointestinal permeability, dual energy x-ray densitometry

66 Reduced bone formation and increased bone resorption drive bone loss in *Eimeria* infected broilers.

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Coccidiosis is one of the most economically important diseases in the global poultry industry. It can cause intestine damage, lead to malnutrition and immune system activation, resulting in poor growth in chickens. However, little is known about the mechanism of bone defects caused by

coccidiosis. To investigate this, a total of 400, day-old male Cobb 500 broilers were randomly allocated into 4 treatment groups: 1) an uninfected control consuming *ad libitum* diet; 2) a minor infected group; 3) a severely infected group; 4) and an uninfected pair-fed group consuming the same amounts of feed eaten by the severely infected group. Both infected groups were orally gavaged with different concentrations of sporulated *Eimeria* oocysts (1.25×10^4 oocysts of *E. maxima*, 1.25×10^4 of *E. tenella* and 6.25×10^4 of *E. acervulina* for minor infection; 5×10^4 oocysts of *E. maxima*, 5×10^4 of *E. tenella*, and 2.5×10^5 of *E. acervulina*/mL for severe infection) on day 14. Growth performance was recorded from day 14 to 28. Blood serum, leg bone, and bone marrow were collected on day 21. Chicken bone parameters were analyzed by μ CT, blood serum was collected for the bone resorption parameters, and bone marrow was used for the expression of key osteogenic and osteoclastogenic genes by real-time RT-PCR. Histological analyses including calcein labeling and tartrate-resistant acid phosphatase staining (TRAP) were used for the detection of bone formation and resorption rate, respectively. Body composition was measured by dual-energy x-ray densitometry on day 28. One-way ANOVA was performed, and means were compared by Tukey's test. Birds infected with a high dose of *Eimeria* displayed a decrease in growth performance ($p < 0.05$), feed intake ($p < 0.05$), fat content and fat percentage ($p < 0.05$), bone mineral content ($p < 0.05$) and bone density ($p < 0.05$), compared with the control group. Calcein labeling and mRNA expression also revealed that *Eimeria* infection significantly decreased osteogenesis and bone growth rate, indicating suppression of bone formation. Meanwhile, TRAP staining and serum RANKL ELISA assay ($p < 0.05$) showed an increase in bone resorption. Interestingly, by comparing RANKL/OPG ratio, different levels of coccidiosis infection showed dissimilar effects on bone growth and bone resorption, indicating that different mechanisms might be involved in regards to bone remodeling status. Taken together, our studies demonstrated that coccidiosis-induced bone loss in broilers is associated with both decreasing bone growth and increasing bone resorption, providing a better insight into bone remodeling during internal parasitic infections in poultry.

Key Words: bone health, bone resorption, *Eimeria*, broilers, osteoimmunology

67 Effects of different methionine to cysteine ratios on the growth performance, gut health, and body composition of broilers challenged with *Eimeria* spp.

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This study was conducted to investigate the effect of different methionine (MET) to cysteine (CYS) ratios in the

growth performance, gut health, and body composition of broilers challenged with *Eimeria* spp. A total of 720 fourteen-day-old male broilers were randomly distributed in a 5×2 factorial arrangement (6 replicates/ 12 birds). Five MET: CYS ratios (T1,100:0; T2, 75:25; T3, 60:40; T4, 25:75; and T5, 0:100) and two challenges (PBS and mixed *Eimeria* spp.) were the main effects. On day 14, the challenged group (CG) was gavaged with 25,000 *E. maxima*, 25,000 *E. tenella*, and 125,000 *E. acervulina* sporulated oocysts, and the non-challenged group (NCG) was gavaged with PBS. Daily feed intake was recorded after challenge. On 5 day post-infection (DPI), intestinal permeability was measured. On 6 and 9 dpi, growth performance was recorded. On 9 dpi, the body composition was determined by dual energy x-ray absorptiometry (DEXA). Data were analyzed by two-way ANOVA and Duncan's multiple range test. The NCG had significantly better ($P<0.01$) growth performance than the CG. The body weight (BW), feed intake (FI), and body weight gain (BWG) linearly decreased ($P<0.01$), and feed conversion ratio (FCR) increased ($P < 0.01$) in the NCG as the MET: CYS ratio decreased on both 6 and 9 dpi. For the CG, on dpi6, there was no significant difference in growth performance among treatments. On dpi 9, only BW, FI, and BWG of T5 were significantly lower ($P<0.01$) and the FCR was higher ($P < 0.01$) than the other groups. There were significant interactions of main effects ($P<0.05$) for BW, FI, and BWG on both time points. For the body composition, the NCG had significantly higher ($P<0.01$) bone mineral density (BMD), bone mineral content (BMC), fat, and muscle percentage than the CG. There was no significant difference in BMD in the NCG. However, in the CG, the BMD is significantly higher ($P<0.01$) in T4 compared to T1 and T3. In the NCG, T2 had higher ($P<0.01$) BMC than T4 and T5, while in the CG, T5 had significantly higher ($P<0.01$) BMC compared to T1. The fat and muscle percentages of the NCG did not differ among treatments. In the CG, T5 had significantly higher ($P<0.01$) fat percentage but lower ($P<0.01$) muscle percentage than T3. In conclusion, the growth performance was adversely affected as the MET: CYS ratio decreased while the body composition was largely unaffected when the broilers were not infected with *Eimeria*. The impact of changing MET: CYS ratio on growth performance was gone or less severe while a significant effect on body composition was observed during the infection period of *Eimeria*, suggesting that MET: CYS ratio plays critical roles in modulating body composition and nutrient partitioning during coccidiosis.

Key Words: Methionine, cysteine, total sulfur amino acid, *Eimeria*, DEXA

68 Reassessing the effects of *Eimeria* spp. infection on broiler performance. Alexandre B. Mariani*^{GS 1}, Catiane Orso¹, William Lambert³, Pierre Gaignon², Helene Pastorelli², Ines Andretta¹ ¹*Animal Science, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil*, ²*École Supérieure d'Agriculture d'Angers, Angers, France*, ³*METEX NOOVISTAGO, Paris, France*.
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Eimeria infections compromise animal welfare and reduce the productivity of poultry sector. This meta-analysis was developed to reassess the effect of *Eimeria* spp. on broiler growth performance. Methods of systematic review were used to access studies published in scientific journals from 2000 to 2020 reported performance of broilers from 1 to 50 days old subjected to an experimental challenge by *Eimeria* spp. (excluding vaccines). Data of 131 articles (162 experiments and 46,354 birds) were included in the database (1,970 lines) and coded to limit the studied effect to the *Eimeria* inoculation (excluding any special condition, such as medication or feed additive supplementation). Results were analyzed as raw data, when control and challenged groups were compared by variance analysis using mixed models with fixed effect of challenge and random effect of study. The percentage of response to the challenge was calculated ($\Delta\%$) and the relationship between the variations in average daily feed intake (ADFI) ($\Delta\%FI$) and average daily gain (ADG) ($\Delta\%G$) was studied by correlation and regression analysis. Broilers challenged by *Eimeria* spp. reduced ADFI by 6%, ADG by 20%, and gain to feed (GF) by 11% ($P<0.001$). Whatever the type of challenge (whole database, type of *Eimeria* species, or pool of species) showed highly significant effects. Only the challenge by *E. maxima* did not influence ADFI. The greatest impairment in broiler performance was found for pool challenge in case of ADFI (-9%) and for *E. maxima* in ADG (36%) and GF (15%) responses. The $\Delta\%G$ showed a linear relationship with $\Delta\%FI$. The correlation between $\Delta\%G$ and $\Delta\%FI$ caused by *Eimeria* challenge was high ($r=0.705$; $P<0.05$). When simulating a scenario without changes in ADFI (i.e., $\Delta\%FI$ was set to zero in the equations), the $\Delta\%G$ was estimated to be 6% for the overall database, 4% in challenges caused by *E. acervulina*, 17% for *E. maxima*, 4% for *E. tenella*, and 5% for pool of *Eimeria*. These reductions represented 32% of total ADG impairment for the overall database, 23% for *E. acervulina*, 65% for *E. maxima*, 18% for *E. tenella*, and 26% for pool of *Eimeria* spp. These results are very important since the relationship between ADFI and ADG impairment are very difficult to quantify in conventional experimental designs. The provided information are also useful to further understand the effects of health challenges in the nutritional requirements used for animal maintenance.

Key Words: coccidiosis, challenge, feed intake, meta-analysis, weight gain

69 Cloning bile salt hydrolase to deconjugate bile acids for potentially intervening necrotic enteritis. Tahrir Alenezi*^{GS 2, 1}, Ying Fu², Ayidh Almansour¹, Hong Wang¹, Xiaolun Sun¹; ¹*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*, ²*Cell and Molecular Biology, University of Arkansas, Fayetteville, Arkansas, United States*.

Huge economic loss in the poultry industry is caused by *Clostridium perfringens* and *coccidia* infection-induced necrotic enteritis (NE). Dietary deoxycholic acid (DCA) of

a secondary bile acid reduces NE, but with the accumulation of less effective conjugated tauro-DCA (TDCA). Bile salt hydrolase (Bsh) produced by gut microbiota catalyzes deconjugation of bile acids. We hypothesized that Bsh increased deconjugated DCA to enhance NE reduction. In this study, based on literature and NCBI database search, bacteria expressing Bsh were examined with DCA precipitation assay using TDCA (0.3, 0.2, or 0.1%) plates at pH 6 supplemented with 0.01, 0.02, or 0.03% CaCl₂. The *bsh* genes were PCR-amplified and Sanger-sequenced. The *bsh* genes were then cloned into protein expression plasmid pET 28a as pET-bsh. After transformed pET-bsh into *E. coli* DH5a and BL21 for amplification and protein expression, Bsh protein was His-tag purified and analyzed by Dot-Blot, SDS-PAGE, and DCA precipitation assays. As a result, we have identified Bsh-expressing bacteria, including two non-pathogenic bacteria *Bifidobacterium longum* and *Lactobacillus johnsonii*. *L. johnsonii* and *B. longum* anaerobically grew colonies surrounded with precipitation zones on plates with 0.2% TDCA and 0.03% CaCl₂. Interestingly, no precipitation zone around colonies was observed when *L. johnsonii* grew aerobically. The *bsh* genes from both *L. johnsonii* and *B. longum* had a similar length of around 1000 bp. Upon Sanger-sequenced the PCR products, the sequences were confirmed the same as in the NCBI database and slightly different between each other. After transformed and expressed pET-bsh in BL21, Bsh protein was isolated using a His-tag purification kit. Bsh was verified by Dot Blot assay, showing darker black dot from the expressed samples compared to light black dot from the control sample. In addition, Bsh was subjected to SDS-PAGE, and Coomassie Blue staining showed strong bands around 32 kDa, the same size as Bsh. To evaluate the function of the expressed Bsh, the protein was examined on plates with 0.2% TDCA and 0.03% CaCl₂, showing crystallized DCA in the path of Bsh solution compared to nothing in elution buffer only. Furthermore, TDCA in the presence of the Bsh reduced *C. perfringens* growth *in vitro* compared to without Bsh. We are cloning the *bsh* genes into *Bacillus subtilis* for chicken experiments. In conclusion, *bsh* genes from *L. johnsonii* and *B. longum* were functional to deconjugate bile acids and enhanced TDCA to reduce *C. perfringens* *in vitro* growth. Bsh proteins have the potential to enhance NE prevention in chickens fed DCA diet.

Key Words: necrotic enteritis, BSH, *Bifidobacterium longum*, *Lactobacillus johnsonii*, taurodeoxycholic acid

70 Comparative efficacy of modern grain sorghum for the control of necrotic enteritis caused by *Clostridium perfringens* in broiler chickens. Alissa Moritz*^{GS 1}, Brett Lumpkins², Greg F. Mathis², Mireille Arguelles-Ramos¹, William Bridges³; ¹*Animal and Veterinary Sciences, Clemson University, Clemson, South Carolina, United States*, ²*Southern Poultry Feed and Research, Inc., Athens, Georgia, United States*, ³*Mathematical and Statistical Sciences, Clemson University, Clemson, South Carolina, United States*.

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Alternative feedstuffs are of interest to reduce the dependence on corn in poultry diets, thereby, alleviating pressure on corn production and potentially improving animal-production efficiencies. Modern U.S. grain sorghum, which is 99% tannin-free, is one alternative feed ingredient due to its nutritional equivalency to corn. Its abundance of polyphenolic compounds associated with antimicrobial function may have direct impacts for control of common poultry intestinal diseases like coccidiosis and necrotic enteritis (NE). A 28d battery cage study was conducted to test the efficacy of modern grain sorghum varieties (red/bronze, white/tan and U.S. No. 2 yellow) fed to Cobb 500 male broiler chickens (n = 512) and challenged with *E. maxima* and *C. perfringens*. All birds were fed 1 of 4 treatments (corn, red/bronze sorghum, white/tan sorghum or U.S. No. 2 yellow sorghum) and were grouped by challenge method (challenged with *E. maxima*/*C. perfringens* or unchallenged). On day 14, birds in the challenge group were orally inoculated with ~5,000 oocysts of *E. maxima* and on days 19, 20, and 21, birds were given a culture of *C. perfringens* once daily. On day 21, 3 birds were removed, examined, and scored for the degree and presence of necrotic enteritis lesions. Birds (8 birds/cage and 8 cages/treatment) and feed were weighed on days 0, 14, 21, and 28 to calculate average feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR). Data was analyzed as a 2-way ANOVA with factors of treatment, challenge method and their interaction. On day 21, FCR was improved for birds fed standard corn and white/tan sorghum treatments (P<0.05) than those fed red/bronze sorghum, while U.S. No. 2 yellow was intermediate. BWG was highest for standard corn, white/tan and U.S. No. 2 yellow treatments (P>0.05) compared to red/bronze. NE lesions on day 21 were observed in standard corn and white/tan compared to no observable lesions in red/bronze (P<0.05), while U.S. No. 2 yellow treatment was intermediate. No significant differences were observed among treatments for 1-28 d BWG and FI. This study demonstrated that birds fed corn and white/tan sorghum treatments had similar performance. Also, red/bronze grain sorghum reduced intestinal lesions of broilers challenged with *E. maxima* and *C. perfringens*.

Key Words: alternative feedstuff, broilers, coccidiosis, grain sorghum, necrotic enteritis

71 Genomic analysis of avian pathogenic *Escherichia coli* (APEC) isolated from broiler chickens. Priyanka Devkota*^{GS 1}, Sabin Poudel¹, Mark A. Arick II², Chuan-Yu Hsu², Pratima Adhikari¹, Anuraj Sukumaran¹, Aaron Kiess¹, Li Zhang¹; ¹*Department of Poultry Science, Mississippi State University, Mississippi State, Mississippi, United States*, ²*Institute for Genomics, Biocomputing, and Biotechnology, Mississippi State, Mississippi, United States*.

Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis. This disease is responsible for significant economic losses in the poultry industry and is one of the

leading causes of morbidity and mortality in chickens. There is limited information about the virulence genotype of the APEC strain due to its complex and diverse serogroup. The main objective of this study was to characterize the genomic sequences of APEC strains isolated from different organs of broiler. The whole-genome sequence (WGS) was performed using Next Generation Sequencing approaches including Oxford Nanopore long read and Illumina short read sequencing methods. The genome sizes for MS1011, MS1085, MS1118, and MS1274 were 4,881,344 bp, 6,045,026 bp, 5,125,887 bp, and 5,007,825 bp, respectively whereas their number of predicted coding sequences (CDs) were 4,308, 5,405, 4,469, and 4,325, respectively. The antimicrobial resistance genes (AMRs) were analyzed using the Resistance Gene Identifier (RGI) web portal. The results showed that MS1118, MS1274, MS1011, and MS1085 consisted of 12, 15, 23, and 11 resistance genes. All four APEC isolates carried multiple AMRs conferring resistance to tetracycline, fluoroquinolone, and macrolide, which are widely used by the poultry industry. The serotype of MS1118, MS1011, MS1274, and MS1085 was O5:H10, O8:H11, O134:H21, and O178:H11, respectively. Among O-antigen, O5 and O8 are most common in poultry, which helps to determine the pathogenic potential of APEC. The virulence factors (VFs) were analyzed using the Virulence Factor Database and MS1118, MS1011, MS1274, and MS1085, contained 31, 30, 46, and 85 VFs, respectively. Whole-genome analysis of these avian pathogenic *E. coli* isolates from our study disclosed a diverse range in genetic composition, AMRs, serotyping, and virulence genes. This information can be used to discover genetic features that allow us to better understand the role virulence and resistance genes play in colibacillosis, while also allowing for the development of strategies to prevent this disease in the future.

Key Words: Antimicrobial resistance genes, avian pathogenic *Escherichia coli*, poultry, virulence factor, Whole-genome sequencing

72 Molecular characterization of virulence and antimicrobial resistance genes in avian pathogenic *Escherichia coli* isolated from poultry having colibacillosis. Deepa Chaudhary*^{GS 1}, Priyanka Devkota¹, Sabin Poudel¹, Linan Jia¹, Anuraj Sukumaran¹, Wen-Hsing Cheng², Aaron Kiess¹, Li Zhang¹; ¹*Department of Poultry Science, Mississippi State University, Mississippi State, Mississippi, United States*, ²*Department of Food Science, Nutrition and Health Promotion, Mississippi State University, Mississippi State, Mississippi, United States*.

Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis, resulting in significant economic losses to the poultry industry worldwide. Numerous virulence genes are responsible for the presence of extraintestinal pathotypes of *E. coli* in chickens, causing the disease colibacillosis. The role of virulence genes in pathogenesis is not yet clearly understood. Thus, this study aimed to characterize typical virulence factors and antimicrobial resistance patterns of

APEC isolates from broiler chickens affected by colibacillosis. A total of 102 *E. coli* isolates were isolated from visceral organs of chickens with typical lesions of colibacillosis. Samples were cultured on MacConkey agar overnight at 37°C, and suspect bacterial colonies were further isolated on LB agar. *E. coli* isolates were confirmed by polymerase chain reaction (PCR). Positive isolates were screened for the APEC-related genes *iroN*, *ompT*, *hlyF*, *iss* and *iutA*, and *E. coli* isolates containing one or more of these genes were identified as APEC-like strains. Prevalence of tested virulence genes was *iroN* (76.5%), *ompT* (77.5%), *hlyF* (77.5%), *iss* (76.5%) and *iutA* (76.5%). Seventy-one isolates (69.6%) carried all five virulence-associated genes. FumC and FimH based – (CH) typing was conducted by a web tool called CHTyper, and C13:H1507 was observed in 33 isolates (32.4%). Twenty-four antimicrobial resistance (AMR) genes were tested by PCR. The resistance gene against tetracycline (*tetA*) was the most frequently found in 72 isolates (70.6%), followed by aminoglycoside (*aph31A*) in 59 isolates (57.8%) and quaternary ammonium compound (*qacEA*) in 33 isolates (32.4%). Of all the tested heavy metal resistance genes, *arsC* was the most abundant in APEC isolates (88 isolates, 86.3%). The results revealed that 11.8% of APEC isolates carried AMR genes encoding resistance to three or more different classes of antimicrobial agents. In conclusion, APEC strains isolated in this study showed a high prevalence of virulence genes and AMR genes. These findings will help characterize APEC more comprehensively and provide important information to better understand its pathogenesis in poultry.

Key Words: Avian pathogenic *Escherichia coli* (APEC), antimicrobial resistance, colibacillosis, poultry, virulence

73 Inflammatory and antibody responses to intradermally administered autogenous *Salmonella* vaccine isolates and content-matched *Salmonella* lipopolysaccharide. Jossie M. Santamaria*^{GS}, Chrysta N. Beck, Marites A. Sales, Gisela F. Erf; *Department of Poultry Science, Division of Agriculture, University of Arkansas, Fayetteville, Arkansas, United States*.

Information on cellular immune system activities to *Salmonella* vaccines (SV) is limited. Using the growing feather (GF) pulp as a test-tissue, the local inflammatory response to intradermally (i.d.) injected test-material can be monitored in an individual. The objective of this study was to determine the local cellular- and systemic humoral-immune responses initiated by GF pulp injection of two autogenous, heat-killed SV (SV1 and SV2) and *S. enteritidis* (SE) LPS. For this, GF of 14-wk-old Light-brown Leghorn pullets were injected with either SV1, SV2, LPS, or vehicle (oil emulsion); 10 µL per GF, 20 GF per bird, 4 birds per treatment. Injected GFs were collected before (0 h) and at 6, 24, 48, and 72 h post-injection for leukocyte population analysis by fluorescence activated flow cytometry. Heparinized blood was sampled before (0 d) and at 3, 5, 7, 10, 14, 21, and 28 d post GF-pulp injection.

Relative plasma levels (a.u.) of SE-specific IgG, IgM, and IgA were determined by ELISA. There were no treatment differences ($P = 0.663$) for total leukocyte levels (% pulp cells). Leukocyte-infiltration was highest at 6 h, declining gradually to above pre-injection levels by 72 h (main effect means 2.3, 40.7, 28.7, 18.0, and 14.0 % at 0, 6, 24, 48, and 72 h, respectively; SEM = 2.3; $P < 0.001$). There were no differences between SV1 and SV2 in the proportions of heterophils, macrophages, T- and B-cells in the pulp at any time-point. LPS had the highest, and vehicle the lowest, heterophil infiltration. However, vehicle recruited much greater numbers of T- and B-cells than the other treatments ($P < 0.001$). Pulp injection of SV1 or SV2 resulted in SE-specific IgM, IgG, and IgA production, with the highest plasma levels for IgM, followed by IgG and IgA. IgM levels were maximal at 5 d, remained at this level until 15 d for SV2 and 21 d for SV1 before declining. IgG reached maximal levels by 10 d for SV2 and 14 d for SV1 and remained near peak levels thereafter. IgA increased similarly in SV1- and SV2-treated pullets with maximal levels maintained from 7 d to 21 d. For LPS, IgM levels were elevated at 5 d to 14 d, with peak levels lower than those with SV1 and SV2 ($P \leq 0.05$). LPS did not result in SE-specific IgG and IgA. Vehicle did not affect circulating levels of SE-specific IgM, IgG, and IgA ($P > 0.05$). Analyses of samples from two-repeat studies are underway. Simultaneous examination of local inflammatory and systemic humoral responses to a primary i.d. administration of SV or SE-LPS provided important new knowledge about the immunostimulatory effects of bacterial vaccines that may lead to protective-, and in some cases pathological-, responses to intracellular pathogens like Salmonella.

Key Words: Inflammatory response, Antibody response, Light-brown Leghorn, Salmonella, vaccine

74 Primary and recall immune responses to autogenous *Salmonella* vaccine or *Salmonella* lipopolysaccharide administration in Light-brown Leghorn pullets. Chrysta N. Beck*^{GS}, Jossie M. Santamaria, Marites A. Sales, Gisela F. Erf; *Department of Poultry Science, Division of Agriculture, University of Arkansas, Fayetteville, Arkansas, United States.*

Salmonella vaccination is extensively used in poultry management to ensure food safety and bird health. However, information on cellular and humoral immune responses to *Salmonella* vaccines is limited. The objective of this study was to analyze the antibody and cellular responses to primary and secondary *Salmonella* vaccinations. Specifically, 14-wk-old Light-brown Leghorn pullets were vaccinated s.c. with 0.5 mL of autogenous *Salmonella* vaccines (SV1 or SV2), *S. enteritidis* (SE) lipopolysaccharide (LPS), or vehicle (V) to initiate a primary response (4 birds/treatment). Birds received the 2nd vaccination 4-wks later by injecting the respective treatments i.d. into the pulp of growing feathers (GF; 10 μ L/GF, 20 GF/bird). Heparinized blood was collected before (0 d) and at 3, 5, 7,

10, 14, 21, and 28 d after the 1st and 2nd vaccination. Relative SE-specific IgG, IgM, and IgA antibodies in plasma were quantified by ELISA. To examine the local cellular response, GF were collected before (0 d) and at 0.25, 1, 2, 3, 5, and 7 d post-GF injection for leukocyte populations analysis by flow cytometry. Antibody and GF data were analyzed by a two-way ANOVA. For the primary and recall immune responses, SV1 and SV2 yielded the highest SE-specific IgM levels by 10 d and LPS by 5 d. For SV1 and SV2, maximal IgG levels were reached at 14 d and 7 d for the primary and recall responses, with much higher levels observed during the recall response. IgA levels increased with SV1 and SV2 during the primary response but were not affected in the recall response. LPS did not induce IgG or IgA, while V did not stimulate IgG, IgM, or IgA production. There was a time effect ($P < 0.05$) but no treatment effect on leukocyte, heterophil, and macrophage levels (% pulp cells) in injected GF-pulps of sensitized chickens. Leukocyte, heterophil, and macrophage levels were elevated between 0.25 d and 3 d ($P < 0.05$). Among infiltrating leukocytes, lymphocytes were the least abundant with SV1, SV2, and LPS injection. However, V injection resulted in T- and B-cell recruitment with greatly elevated ($P < 0.01$) levels of CD4⁺ and $\gamma\delta$ T cells at 0.25 d post-GF injection. In summary, the quantitative, qualitative, and temporal characteristics of the humoral response to SV1 and SV2, with isotype switch to IgG and a memory phenotype, are as expected with T-dependent antigens. The local inflammatory response profile to *Salmonella* in GF of sensitized chickens differs from that reported for *Mycobacterium butyricum* (Erf & Ramachandran, 2016) where T lymphocytes dominated the infiltration response. Therein, Gram-negative and Gram-positive bacteria generate different cellular immune system activities in an immunized host.

Key Words: Salmonella, Lipopolysaccharide, Antibody response, Inflammation, Leukocyte recruitment

75 Glucosinolate containing ingredients (rapeseed and canola meal) and glucosinolate metabolite (AITC) as potential antimicrobials: Effects on growth performance, and gut health in *Salmonella* Typhimurium challenged broiler chickens. Sudhir Yadav*^{GS}, Po-Yun Teng, Amit Singh, Sasikala Vaddu, Harshavardhan Thippareddi, Woo K. Kim; *Poultry Science, University of Georgia, Athens, Georgia, United States.*

Poultry is the major source of foodborne salmonellosis. Antibiotic resistance and a surge in zoonotic diseases warrant the use of natural alternatives. Glucosinolates (GLs) are naturally occurring antimicrobial compounds in rapeseed and canola. This study investigated the effect of feeding rapeseed, canola meal, and allyl isothiocyanate (AITC; *Brassica* secondary metabolites) on growth performance (GP), gut health and the potential antimicrobial activity against nalidixic acid-resistant *Salmonella* Typhimurium (ST^{NR}) in chickens. A total of 640 one-day old male Cobb 500 broilers were

randomly allocated to 8 treatments with 8 replicated cages and 10 birds per cage. Dietary treatments were non-challenge control (NC, corn-SBM based), challenge (*Salmonella*) control (CC), 10% rapeseed (10RS), 30% rapeseed (30RS), 20% canola meal (20CLM), 40% canola meal (40CLM), 500 ppm AITC (500AITC) and 1,500 ppm AITC (1500AITC). On d 1, all the birds except NC were orally challenged with ST^{NR} (ca. 7 log CFU/ bird). The chickens were reared for 21 d, and their FI and BW were recorded weekly. *Salmonella* cecal colonization and fecal shedding were quantified whereas organ translocation (OT) of ST^{NR} to the spleen, liver, and kidney was tested (1 bird/cage) on 0, 3, 6, 13, and 20-d post-challenge (dpc). Data were subjected to one-way ANOVA and the means were separated by Duncan's test, except mortality and OT data analyzed after transformation by square root of (n + 1) ($P < 0.05$). Overall, feeding 30RS resulted in reduced BW ($P = 0.003$), BWG ($P = 0.003$), and FI ($P = 0.001$) compared to CC, 500AITC, and 1500AITC. Similarly, feeding 20CLM resulted in lower BW and BWG compared to CC ($P < 0.05$) and increased FCR compared to 1500AITC ($P = 0.03$). Feeding CC resulted in higher mortality compared to NC and 30RS ($P = 0.03$). Cecal colonization of ST^{NR} was reduced ($P < 0.0001$) for 30RS on 6 dpc and 500AITC on 6- and 13 dpc ($P < 0.0001$). Although no difference in gut permeability was observed 6 dpc ($P > 0.05$), OT of ST^{NR} population was the highest for CC in the spleen ($P = 0.05$). In the liver, 10RS showed reduced OT compared to 20CLM on 13 dpc ($P = 0.03$) whereas in the kidney, 30RS showed the lowest OT on 6 dpi. Fecal shedding was lowest for 30RS on 6 dpc ($P = 0.004$). Histomorphology showed 30RS had the highest duodenum ($P = 0.01$) and jejunum ($P = 0.02$) villus height (VH) and VH to crypt depth (CD) ratio compared to other treatments whereas 1500AITC showed similar results to 30RS. Both 30RS and 1500AITC contained comparatively higher functional GL metabolites and were able to maintain gut health. Including higher levels of rapeseed or AITC in poultry feed can reduce *Salmonella* populations in the feces and translocation to other organs.

Key Words: glucosinolate, antimicrobial, gut health, Brassica species, chickens

76 Horizontal transmission of histomoniasis may be influenced by feed composition and strains of *Histomonas meleagridis*. Thaina L. Barros*^{GS 1}, Christine Vuong¹, Elizabeth McGill², Samuel J. Rochell¹, Guillermo Tellez-Isaias¹, Billy M. Hargis¹; ¹Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, ²Cargill Turkeys LLC, Springdale, Arkansas, United States.

Histomoniasis is spread by direct contact within turkey flocks. Experimental reproduction of horizontal transmission (HT) has not been reliable during recent decades. In our area, pre-breeder turkey hens, fed low-nutrient-density diets, are most affected by *Histomonas meleagridis* (HM) infections. We compared two strains of

HM and two low-nutrient-density diets, varying in ingredients and nutrient composition, on HT of HM. In exp. 1, day-of-hatch poult were randomly distributed to either a non-challenged control (NC; n=34) or HT group (n=34; 10 of which were directly challenged - seeders). Intraloacal challenge was performed with a strain of HM (PHL) on day 18 (10^5 histomonads/poult); poult were fed a low-nutrient-density diet with high levels (20%) of wheat middlings (WM). In exp. 2, two low-nutrient-density feeds were compared after the starter diet (d1-7): a similar WM diet as used in exp. 1 (d7-38) or a corn-soy based diet (CS). Two strains of HM were tested: Buford (Bu) or PHL. Day-of-hatch poult were randomly distributed to one of six groups: non-challenged, CS diet (CS-NC); non-challenged, WM diet (WM-NC); HT with Bu strain and WM diet (Bu-WM); HT with Bu strain and CS diet (Bu-CS); HT with PHL strain and WM diet (PHL-WM); or HT with PHL strain and CS diet (PHL-CS). On day 10, 14/45 poult in the HT groups were directly inoculated with 10^5 HM/poult. Mortality was recorded daily in both experiments. Lesion scores were analyzed using PROC MIXED procedure (SAS 9.4). Mortality was compared using the chi-square test of independence. In exp. 1, mortality of the seeder turkeys started on day 39 (21 d.p.i.). On day 44 (26 d.p.i.), all seeders were humanly euthanized and one contact turkey died. The exp. was terminated on day 52 (34 d.p.i.) and lesions were scored on a scale of 0-3. From the contacts, 33% had lesions in the ceca and/or liver (7/23). In exp. 2, mortality started 10 and 11 d.p.i. in the seeder turkeys in the groups Bu-CS and Bu-WM, respectively, and 16 and 21 d.p.i. in the groups PHL-WM and PHL-CS, respectively. Mortality of the seeder turkeys was 42.9% in the PHL-CS and PHL-WM groups, 57.1% in Bu-CS, and 64.3% in Bu-WM ($p > 0.05$). On day 38 (28 d.p.i.), all groups were terminated and lesions in the liver and ceca were evaluated. In exp. 2, HT was only observed in the PHL-CS, with 23.3% mortality in the contact turkeys and 57% of the contacts exhibiting lesions. In summary, HT was reproduced in two experiments using low-nutrient-density diets, although the WM diet did not allow successful HT in exp. 2, suggesting that either subtle differences in diets or infrequent high-level HM shedding from seeders are responsible for HT of HM. Ongoing investigations will examine these hypotheses.

Key Words: Blackhead disease, horizontal transmission, turkeys

77 Role of stress factors on histomoniasis disease development and transmission in turkeys. Catherine Fudge*^{GS}, Chongxiao Chen, Robert Beckstead; *Prestige Department of Poultry Science, NC State University, Raleigh, North Carolina, United States.*

Histomonas meleagridis infection leads to cecal and liver lesions, severe morbidity, and high mortality in turkeys. Few studies have researched the stress factors involved in histomoniasis development and transmission. Two studies were conducted to investigate the role of common stressors during turkey production on histomoniasis development and

transmission between poult. Experiment 1 (floor pen trial): 480 one-day-old poult were allocated to 8 treatment groups having 3 replicates of 20 birds each. Treatments consisted of negative control, positive control, cold stress (15.6 C, for 12 days post-infection (DPI)), 18-hour feed withdrawal (1 DPI, 6 AM-12 PM), transportation stress (1 DPI transported 2 hours), poor poult quality (24 hour delayed placement), increased electrolyte diet (3.5% increase), feed additive (butipearl). At 5 weeks, 10 birds in each pen were infected cloacally with 100,00 histomonads/birds, except negative control pens. The trial was terminated 35 DPI. Experiment 2 (battery cages trial): 130 one-day-old poult were allocated to 4 treatment groups having 3 replicates of 10 birds, and 1 negative control cage. Treatments consisted of negative control, positive control, low protein diet (8% reduction), low protein diet, and 5X vaccine dose consisting of *Eimeria adenoidis* and *Eimeria meleagridis*, low protein diet, and 18-hour feed withdrawal (1 DPI, 6 AM-12 PM). At 2 weeks, 5 birds in each treatment cage were infected cloacally with 100,000 histomonads/bird, except negative control cages. Poult were terminated 28 DPI. Daily mortality was recorded throughout experimental periods. Cecal and liver lesions caused by *H. meleagridis* infection were scored on a 0-4 scale with 4 being the most severe. Data were analyzed using the GLM procedure using SAS (9.4) and means were separated by Duncan Multiple Range Test with a significant difference being $P \leq 0.05$. Significant transmission of *H. meleagridis* from infected to uninfected birds was not observed during either experiment. In experiment 1, 18-hour feed withdrawal and electrolyte treatments had significantly higher cecal ($P=0.0094$) and liver ($P=0.0046$) scores compared to the rest of treatments. 18-hour feed withdrawal treatment had highest mortality, followed by salt treatment ($P=0.00470$). There is no significant difference in mortality between the rest of treatments. In experiment 2, 5X vaccine and 18-hour feed withdrawal treatments had significantly higher mortality than positive control group ($P=0.0243$); ceca and liver lesion scores between treatments were not significantly different. Among the factors tested, feed withdrawal, high electrolytes, and co-infection with 5X cocci vaccine resulted in more severe disease signs at termination.

Key Words: histomonas, blackhead, transmission, stress, parasitology

78 Fructans affects mRNA expression of pro-inflammatory, antiviral, and antibacterial innate immunity genes on chicken macrophages. Santiago Uribe-Diaz*^{GS 1}, Blanca C. Martinez², Julian Reyes¹, Juan Carlos Rodriguez-Lecompte¹; ¹University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada, ²Promitec S.A, Bucaramanga, Colombia.

A study was conducted to evaluate the effects of fructans-like fructooligosaccharides (FOS) and inulin supplementation on innate immune activation in chicken macrophages. **Methods:** Chicken macrophages (HD-11) cell line were cultured by triplicate at a concentration of

5×10^5 cells / well in 24 well plates. Macrophages were divided into three groups: 1. FOS 54.7 ug/100 ul powder; 2. FOS syrup 1 ug/100 ul; and 3. Inulin 35.3 ug/100 ul. This study evaluates periods of time 12- and 24-hours post-supplementation. The research tested the effect of FOS powdered or in syrup and inulin supplementation on chicken macrophages gene expression of toll-like receptors (TLR) 2, 4, 3 and 21; macrophage mannose receptor (MMR); adapter molecules TRIF and MyD88; transcription factor NF- κ B, interferon regulatory factor-7; pro-inflammatory cytokines interleukin (IL)-1b, IL-6, IL-12, IL-17, IL-22 and IFN-g; anti-proinflammatory cytokines TGF- β , IL-4, IL-10; antiviral interferon (IFN) type I- β ; and antimicrobial ribonuclease angiogenin 4 (Ang-4). They were used as indicators of immunological cell activation. Gene mRNA expression was quantified in chicken macrophages using quantitative RT-PCR with SYBR green dye. An ANOVA model was used to compare the effects of treatment groups, genes, and time on the natural logarithm of Ct values; a pairwise comparison of marginal predictions for the treatment groups were performed using Tukey's adjustment. **Results** TLR-2 and MMR were not significantly upregulated or downregulated ($P > 0.05$) at any time or treatment group. However, TLR3, TLR4, and TLR-21 were significant different ($P < 0.05$). TRIF was significantly upregulated during the first 12 h of supplementation in the group 1; in contrast, MyD88 and NF- κ B shown no differences ($P > 0.05$) between groups. IRF-7 was upregulated in group 1 at both sampling times. Expression of IL-1b and IL-6 were upregulated ($P < 0.05$) at 12 h in group 1, in contrast to IL-12 upregulation ($P < 0.05$) at 24 h. However, IFN-g was upregulate in groups 2 and 3 in both at 12 and 24 h. IL-10 was significantly upregulated ($P < 0.05$) at 12 h. Ang 4 was upregulated in group 1 at 12 h. **Conclusion:** Fructans-like fructooligosaccharides and inulin supplementation have been shown to improve microbial balance of the intestinal ecosystem by stimulating the growth of bifidobacterial, lactobacilli and Bacteroides. However, the supplementation with Fructans was shown to activate the endosomal patterns recognition receptors that modulate the pro- and anti-proinflammatory balance in chicken macrophages.

Key Words: Innate immunity, Macrophages, FOS, Health, Fructans

79 Fecal and serum biomarkers for intestinal inflammation in broiler chickens. Gabriela Cardoso Dal Pont*^{GS 4}, Bruna L. Belote¹, Christos Gougoulis², Yuhua Z. Farnell⁴, Michael H. Kogut³; ¹Veterinary Sciences, UFPR, Curitiba, Brazil, ²Innovad NV/SA, Essen, Belgium, ³USDA-ARS, College Station, Texas, United States, ⁴Poultry Science, Texas A&M University, College Station, Texas, United States.

Gut health has been in the focus of the poultry industry for years, specifically driven by the reduction of antibiotics in poultry production. However, to apply measures and products to improve gut health, the industry seeks reliable

non-invasive biomarker(s) to evaluate intestine status of the flocks and guide interventions. Therefore, we conducted an experiment to evaluate several proteins as non-invasive biomarkers for gut inflammation in broilers. We used two models of intestinal inflammation: a chemical model using timed challenges with dextran sulfate sodium (DSS) and a nutritional model using a high non-starch polysaccharide diet. In the present study, 180 day-of-age chickens were randomly divided in four treatments: 1) Control (CNT) with no challenge; 2) two cycles of oral-gavage of 0.25mg DSS/ml (25DSS); 3) two cycles of 0.35mg DSS/ml (35DSS); or 4) a high non-starch polysaccharide diet (NSP) (30% of rice bran). To confirm if the nutritional and/or the chemical challenges produced an intestinal inflammation histologic analyses were performed at 14, 22, 27 and 36d post-hatch. The proteins assessed as potential biomarkers were: calprotectin (CALP) and lipocalin (LCN-2) in the feces, moreover ovotransferrin (OVT), and CALP, LCN-2 and LPS were measured in the serum. All the proteins were quantified with ELISA kits. Data analysis considered the completely randomized design, variables with non-normal distribution were analyzed by Kruskal Wallistest, normal distribution data were submitted to ANOVA and means were compared by Tukey and Dunnet's, and $P < 0.05$ was considered statistically significant. The three challenged groups presented signs of inflammation under histologic evaluation as an increase in lamina propria and epithelial thickness, infiltration of inflammatory cells, congestion, and proliferation of immature enterocytes. CALP concentration in the serum was significantly greater in 35DSS group by 135% compared to CNT at 14d ($P = 0.036$). Calprotectin was the only serum biomarker showing responsiveness to inflammation. Among the proteins analyzed in the feces, CALP and LCN-2 showed statistical differences at 28d. The 35DSS broilers showed a greater fecal CALP concentration when compared to the CNT by 24.99% ($P = 0.0395$). LCN-2 was 27.09% higher on the feces of NSP broilers than the CNT ($P = 0.0075$). OVT and LPS were not observed as potential biomarkers. Thus, LCN-2 may be a promising fecal biomarker for intestinal inflammation, but most importantly, calprotectin showed the greatest potential as a novel biomarker for poultry measured both in serum and feces.

Key Words: calprotectin, biomarker, lipocalin, intestinal health, gut inflammation

80 Comparison between the I See Inside methodology and measurement of intestinal villi in broilers. Bruna L. Belote*^{GS 1, 2}, Igor Soares¹, Béatrice Hamel³, Cleverson de Souza⁴, Elizabeth Santin^{1, 3}; ¹Department of Veterinary Science, Federal University of Parana, Curitiba, Brazil, ²ISI Institute, Curitiba, Brazil, ³Jefo Nutrition Inc., Sant-Hyacinthe, Quebec, Canada, ⁴CLS Consulting, Capanema, Parana, Brazil.

The extension of the villi surface is positively associated with its absorptive capacity, so the classic morphometry of the villi has become a traditional tool in animal research.

However, this measurement may not be enough to express all the alterations related to mucosa functionality. The aim of this study was to compare the classic morphometry of the intestinal villi with the I See Inside (ISI) scoring method. Therefore, 720 male broilers were raised from 1 to 35 days of age and randomly assigned to the groups non-challenged (NC) and challenged with *Eimeria* (CH), with 24 pens per group. At 17d, the CH birds received an in-feed inoculation with 2×10^5 oocysts isolated in the field. The diet was according to NRC. At 22d, 12 birds per group were necropsied for jejunum sampling and histological analysis, with both villi morphometry and ISI scoring performed on the same villi. The villi morphometry was composed by the measurement of villus height (VH), villus width (VW), crypt depth (CD), crypt width (CW), and the VH:CD relation. For the ISI scoring, the evaluated parameters were lamina propria thickness (LPT), epithelial thickness (ET), proliferation of enterocytes (PE), inflammatory cell infiltration in the epithelial (IFEP), inflammatory cell infiltration in the lamina propria (IFLP), increase of goblet cell (GC), congestion (CG) and presence of *Eimeria* (OOC). For ISI, each parameter was scored (S) from 0 to 3, according to the extension and intensity, which were multiplied by the parameter impact factor (IF), which varies from 1 to 3 according to the impact in the villi function. The final ISI score is $= \sum (IF * S)$. Data were submitted to the Kruskal-Wallis test ($P < 0.05$). The Spearman correlation's test was performed to observe the interaction between the ISI scores with the measurement of the villus ($P < 0.05$). The CH group presented a higher ($P < 0.01$) ISI total score in comparison to the NC group (16 vs 5.9, respectively) due to LPT, IFEP, IFLP and OOC. The VH and VH:CD were lower in the CH group ($P < 0.05$), but the VW, CD, and CW measurements were higher compared with the NC group ($P < 0.05$). The VW had positive correlation with LPT ($r = 0.59$), ET ($r = 0.68$), PE ($r = 0.70$), IFEP ($r = 0.56$), IFLP ($r = 0.50$) and ISI total ($r = 0.62$). The results suggest that the broader contact surface in infected villi may result from the increased inflammation by the *Eimeria* challenge, which may reduce the intestinal absorption function. In conclusion, a larger contact surface is not necessarily related to better intestine function, since it might be compromised by inflammatory cell proliferation. The histopathologic scoring is important to detect biologic differences in treatment groups, providing more information about gut health.

Key Words: Histopathology, Gut health, Inflammation, Scoring method, Index

81 Changes in gene expression in the intestinal mucus of broilers with woody breast myopathy. Linan Jia*^{GS 1}, Chuan-Yu Hsu², Aaron Kiess¹, E. David Peebles¹, Wei Zhai¹, Li Zhang¹; ¹Department of Poultry Science, Mississippi State University, Mississippi State, Mississippi, United States, ²Institute for Genomics, Biocomputing, and Biotechnology, Mississippi State University, Mississippi State, Mississippi, United States.

Woody breast is a breast muscle myopathy in fast-growing broilers related to oxidative stress, gut barrier condition, and inflammation. Mucus that covers and protects the gastrointestinal tract is a dynamic system regulating oxidative stress, gut barrier function, and immune reactions. Previous work has shown that different dietary treatments affect woody breast incidence differently, indicating that gut condition changes are likely related to woody breast myopathy. In this study, the effects of dietary supplementation of antibiotics (bacitracin) or a probiotic (*Bacillus subtilis*) on gene expression related to oxidative stress, the gut barrier function, and inflammation in the mucus lining the jejunum of broilers with or without woody breast myopathy was investigated. A split-plot design was used. The dietary treatments served as a main plot factor with a pen as the main plot unit; woody breast myopathy serves as a subplot factor, with a bird as the subplot unit. Pens were blocked based on their locations. On d 41, jejunum mucus was collected from one bird from each pen exhibiting woody breast myopathy and an additional bird exhibiting normal breast (4 replicates/treatments, total N = 24). Total RNA was extracted using a commercial RNA extraction kit. The expression level of interferon-gamma (IFN- γ), Interleukin 10 (IL-10), Toll-like receptor 2A (TLR2A), Claudin-1 (CLDN1), superoxide dismutase (SOD), and Mucin 6 (MUC6) were determined by two-step reverse-transcription quantitative PCR (RT-qPCR) analysis, and the gene expression difference was determined using the relative quantification ($\Delta\Delta C_t$) method after normalizing with the reference gene (chicken 18S rRNA gene). The fold change of each gene was analyzed using two-way ANOVA, and the level of significance was set at $p \leq 0.05$. There were no significant interactive effects between diet and incidence of the woody breast on any gene expression. The expression of SOD decreased in birds fed antibiotic diet compared to birds fed control or probiotic diet ($p < 0.0001$). Diet modulation did not affect the expression levels of other genes. Birds with woody breast myopathy showed lower MUC6 gene expression levels than birds with normal breast muscles ($p = 0.0009$). No significant expression differences were found between birds with woody breast myopathy and birds with normal breasts on the other genes. In conclusion, broiler woody breast myopathy is related to the decreased expression of mucin in mucus, indicating a correlation between woody breast incidence and a lessening of gel-forming mucin secretion, as well as a reduction in gut defense function.

Key Words: Bacitracin, *Bacillus subtilis*, gene expression, breast muscle myopathy, broiler

82 Impact of deoxynivalenol on intestinal explants of broiler chickens: an *ex vivo* model to assess the efficacy of an anti-mycotoxin additive. Cristina T. Simões*^{GS 1}, Vinícius Duarte¹, Franciéli A. Molossid², Diogo Liberalesso¹, Eduarda D. Gubiani¹, Thaís D. Moreira¹, Cíntia Londero¹, Carlos A. Mallmann¹; ¹*Department of Preventive Veterinary Medicine, Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Poult. Sci. 100 (E-Suppl 1)*

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The impact of deoxynivalenol (DON) upon intestinal tissue of broilers was assessed by using an Ussing Chamber (UC) with jejunal explants associated with histopathological and immunohistochemical measurements. This method was also applied to evaluate the efficacy of an anti-mycotoxin additive (AMA) against DON effects in the intestinal tissue. A total of 12 Cobb 500 male broilers were reared in battery cages and fed a common diet free of aflatoxins, fumonisins, zearalenone, deoxynivalenol, and T-2 toxin contamination. At day 21, all birds were slaughtered and four jejunal explants were immediately obtained from each bird ($n=48$) for the *ex vivo* analysis in the UC. The 48 explants were mounted on UC and subjected to four treatments with 12 replicates in a completely randomized block design, where each bird was considered as block. Treatments were: T1 (control) - buffersolution (BS) without DON and AMA, T2 - BS + 10 mg/L DON, T3 - BS + 0.5% AMA, and T4 - BS + 10 mg/L DON + 0.5% AMA. After 120 min of exposure in the UC, explants were removed and prepared for the following histopathological and immunohistochemical measurements: villi number, villi height, crypts diameter, villi height/crypts diameter ratio, height of apical enterocytes and size of their nuclei, scores of villi integrity and cytoplasmic vacuolization of enterocytes, and number of apoptotic cells. Statistical analyses were conducted using the software Statgraphics Centurion XV. Data were submitted to analysis of variance and mean differences among treatments were compared by Tukey test ($P \leq 0.05$). Villi number, villi height, crypts diameter and villi height/crypts diameter ratio were not different ($P > 0.05$) among treatments. When compared to the control treatment, DON triggered a reduction ($P < 0.05$) on enterocytes size and enterocytes nuclei size as well as an increase ($P < 0.05$) in the scores of cytoplasmic vacuolization microvilli integrity. Apoptotic cells count was greater ($P < 0.05$) in DON-exposed explants compared to the control. It was observed that the AMA mitigated DON harmful effects, where BS + 10 mg/L DON + 0.5% AMA had lower enterocytes height than the control; however, it was higher than BS + 10 mg/L DON ($P < 0.05$). The AMA also preserved the size of enterocytes nuclei, promoted a decrease ($P < 0.05$) in the score of cytoplasmic vacuolization of enterocytes and in the apoptotic cells count. The *ex vivo* model in association with histomorphometry demonstrated the impact of DON upon intestinal explants of broilers as well as the efficacy of an AMA in combating some damaging effects on tissue integrity.

Key Words: Anti-mycotoxins additive, broiler, histopathology, intestinal integrity, Ussing chamber

83 Role of autophagy machinery dysregulation in bacterial chondronecrosis with osteomyelitis. Alison Ferver*^{GS 2}, Elizabeth S. Greene¹, Sami Dridi¹; ¹*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, ²Cell and Molecular Biology, University of*

Arkansas, Fayetteville, Arkansas, United States.

Autophagy has proven to be a significant mechanism for cell survival as well as death. The conflicting nature of the autophagy mechanism means its function or dysfunction has been implicated in both disease states and physiological maintenance. In the case of bacterial infections, it is both a mode of defense via foreign identification and degradation and a means of bacterial proliferation and consequential cell death via manipulation or repression of autophagy machinery. In regards to physiological maintenance, autophagy is critical to proper cell differentiation and proliferation in bone during both growth and healing. It is for these reasons we hypothesized that autophagy could play a role in the pathogenicity of bacterial chondronecrosis with osteomyelitis (BCO). To test this hypothesis, bone samples were taken from non-lame, male broilers raised on clean, shaved-wood litter exhibiting no BCO lesions and lame, male broilers reared on the wire floor model developed by Wideman et al. 2012 exhibiting BCO lesions (n=10/group). The samples were snap frozen in liquid nitrogen and stored at -80°C until further analysis. Gene expression of healthy and BCO-affected birds was analyzed via real time quantitative PCR. Data were analyzed by one-way ANOVA or Student's t-test, as appropriate. Significance was set at $P < 0.05$. To test whether bacterial manipulation was the cause of autophagy dysregulation, human fetal osteoblast cells (hFOB) were challenged with the BCO causing strain *Staphylococcus agnetis* 908. Cells were grown to 80% confluency, treated with *S. agnetis* 908 at an MOI of 50:1, along with untreated controls.. Protein from both cells and tissue was analyzed via Western Blot analysis on 10% acrylamide gels. Image acquisition and analysis were performed by AlphaView software. BCO affected bone showed decreased mRNA and protein expression of key autophagy markers such as LC3, ATG3 and ATG7. Challenged hFOB cells also showed decreased cell viability and decreased protein expression of autophagy machinery and regulators. Global knockdown of autophagy via two different pharmacological inhibitors, 3-Methyladenine and Chloroquine, resulted in similar cell viability decrease as seen in bacterially challenged cells. These results suggest that bacterial infection induces bone attrition via dysregulation of autophagy machinery.

Key Words: autophagy, osteomyelitis, broiler, lameness, bacterial chondronecrosis

84 Generation of chicken anti-human rotavirus recombinant monoclonal antibodies using single B-cell sorting. Jill W. Skrobarczyk*^{GS 1}, Cameron L. Martin¹, Suresh Pillai^{2,3}, Luc R. Berghman¹; ¹*Poultry Science, Texas A&M University, College Station, Texas, United States*, ²*Food Science and Technology, Texas A&M University, College Station, Texas, United States*, ³*National Center for Electron Beam Research, Texas A&M University, College Station, Texas, United States*.

Rotavirus is the leading cause of severe gastroenteritis in infants and young children. One of the primary structural

features of human rotavirus (HRV) is the VP4 spike protein. Cleavage of VP4 by trypsin is required for host cell attachment and subsequent infection. In a previous study, we generated high titer, neutralizing egg yolk anti-HRV antibodies capable of preventing rotavirus infection in vitro. The aim of this study was to generate mono-specific, neutralizing recombinant chicken monoclonal antibodies directed against each of three HRV-neutralizing epitopes found on the VP4 spike protein. We expect that these monoclonal antibodies will exhibit increased HRV neutralizing activity and can be produced in a scalable recombinant antibody expression system. Single B-cell cloning was used to develop the antibody, a technology that has yet to be used in the production of therapeutic monoclonal antibodies in chickens. Peripheral blood mononuclear cells (PBMCs) were isolated from white leghorn hens hyperimmunized with electron beam inactivated HRV particles. Chicken B-cells were sorted by flow cytometry using a fluorescently labeled antibody against chicken IgY and three synthetic, biotinylated peptides derived from neutralizing regions of the rotavirus spike protein. Single B-cells were maintained in vitro in the presence of chicken CD40 ligand and IL-4 for one week before screening for HRV-specific antibody secretion by ELISA. RNA was isolated from positive B-cell clones using the Zymo Mag-Bead RNA Isolation kit and used to generate cDNA. The variable regions of the chicken heavy (VH) and light (VL) chain were amplified using gene specific primers. The VH and VL amplicons were cloned into the Zero Blunt II Sequencing vector for sequence analysis. Once productive VH and VL sequences were obtained, the amplicons were ligated into the pcDNA3.1 vector using a HiFi Assembly kit for expression of the complete antibodies in CHO cells. Three unique antibody sequences incorporating different V-genes were obtained by single B-cell sorting, each directed against one of three spike protein neutralizing epitopes. The antibodies were correctly assembled and expressed for use in future in vitro HRV neutralization studies.

Key Words: Antibody, Monoclonal, B-cell, Sorting, Rotavirus

85 No presentation materials submitted.

86 Evaluation of the trade-off between molt and innate immunity in laying hens. Andrea DeRogatis*^{GS}, Kirk Klasing; *Animal Science, University of California, Davis, Davis, California, United States*.

For poultry, there are a variety of nutritionally costly life stages that must be balanced with the costs of the immune system. The process of molt is known to be expensive in terms of nutrient requirements due to the metabolic costs associated with producing high quality feathers quickly. The trade-offs between investments in self-maintenance (e.g. immunity, antioxidant defenses) and other processes have been shown to be important during both growth and reproduction, but less is known about the relationship

between molt and immunity. The objective of this study was to use chickens (*Gallus gallus domesticus*) to clarify the impacts of molt on nutritional investment in immunity during a lipopolysaccharide (LPS) challenge to the innate immune system. We hypothesized that chickens undergoing molt are likely to have a lower inflammatory response to LPS compared to the other treatment groups. Four treatment groups (n = 7) were used: 1) No molt control 2) No molt control + LPS challenge 3) Molt and 4) Molt + LPS challenge. To induce molt, laying hens were shifted from a long-day schedule to a short-day schedule over the course of a week and administered oral thyroxine at a dose of 1.25 mg/kg of body weight. An intraperitoneal injection of LPS (1.5 mg/kg) was administered three weeks after the onset of molt. Four hours after the LPS injection; liver, spleen, thymus, and blood samples were collected for evaluation of cytokines using qPCR. Analysis of variance (ANOVA) was used to calculate the effect of molt status, body weight and

LPS injection on the spleen or thymus size. Body weight did not affect spleen or thymus size. Molt status was found to significantly affect the size of both the spleen ($p < 0.001$) and the thymus ($p < 0.001$). The average size of the spleen and thymus of a molting chicken was 2.53 and 2.05 grams respectively. The average spleen and thymus of size of a non-molting chicken was 1.48 and 0.41 grams respectively. Counter to our hypothesis, chickens undergoing molt had a stronger inflammatory response to LPS compared to other treatment groups indicated by proinflammatory cytokine expression levels ($p < 0.05$). This research helps clarify how immunity is affected by molt and may be used to inform subsequent research on poultry management practices to optimize immunity during molt.

Key Words: Hens, Molt, Innate Immune System, Immunity, Inflammation

Management and Production

87 Protocol development for monitoring hydrogen sulfide emitted from poultry excreta – a case study. Sam Shen*, Hector Leyva-Jimenez, Katherine McCormick, Michael Martin, Pai Liu; *United Animal Health, Inc., Sheridan, Indiana, United States.*

Noxious odors from animal operations are the result of uncontrolled microbial fermentation of fecal materials. Their impacts can range from unpleasant odors in the environment that will impact animal performance and health, to litigation that could lead to a license revoke and significant financial burden for poultry companies. To counter their harmful impact, versatile remediation approaches including in-feed strategies are commonly used. The effectiveness should be evaluated by odor measurements. However, reliable odor detection protocols that are suitable for field application are lacking. In fact, odor results for ammonia (NH₃) or hydrogen sulfide (H₂S) detected in poultry excreta can be either very variable or not trustworthy for various reasons. While H₂S is more pungent than NH₃, it is a less researched subject. The objective of the current case study was to develop a protocol for monitoring H₂S emitted from broiler excreta applicable to field conditions. The presence and intensity of H₂S from animal excreta rely on the dynamic balance of its formation and reduction, under mostly anaerobic fermentation conditions. A measurement protocol should therefore mimic this process. Based on our experience, predetermined sample preparation was set for the current study. The excreta were conditioned to contain the right amount of moisture and a sufficient amount of sample in a sealed incubation unit (IUT). The samples were incubated for a fixed period under proper temperature before odor measurement. Disposable food containers (1.4L) were used as the IUT, and Dräger® H₂S diffusion tubes (10-300 ppm) as the monitoring tool. The present study was composed of two separate trials with both trials using excreta collected from grower age broilers. The collected excreta were an accumulation of 10-14 days. In the first trial, there were 3 replicate IUT, whereas there were 4 IUT in the second trial. Each IUT replicate had 400-500 g homogenized excreta sample (Trial 2 and 1, respectively) that was conditioned with distilled water before incubation. Excreta in IUT were then incubated for 24 h (25-28°C), before diffusion tube insertion. The H₂S results were expressed as ppm H₂S per 100 g of excreta after 1 h evaluation. Under the current trial conditions, H₂S in excreta collected from birds fed corn-soy-DDGS diets was readily detectable and variation was low (122.7±4.6 ppm/100 g excreta in the first trial and 129.7±21.9 ppm/100 g excreta in the second trial). The protocol developed from the current study allows dynamic and quick H₂S monitoring that will further offer tools for evaluating the effectiveness of feeding strategies in odor remediation.

Key Words: Hens, Molt, Innate Immune System, Immunity, Inflammation

88 In vitro estimation of plant extracts for their antimicrobial activity against *Salmonella Enteritidis*. Weifeng Han, Chun-qi Gao*; *College of Animal Science, South China Agricultural University, Guangzhou, China.*

The addition of antibiotics in livestock and poultry feed will lead to safety problems such as residues. With the coming of the era of non-resistance, it is becoming more and more urgent to find effective substitutes for antibiotics. *Salmonella enteritidis* is one of the typical pathogenic bacteria that target intestinal epithelium, brings huge losses to the poultry industry every year. In recent years, studies have shown that plant extracts can effectively inhibit the growth and reproduction of a variety of pathogens. This study aims to screen the plant extract that can effectively inhibit the activity of S.E. Two experiments were conducted to screen plant extracts that effectively inhibit S.E. In experiment 1, the minimal inhibitory concentration (MIC) and inhibition zone of 10 plant extracts against S.E were tested. The 10 plant extracts contained African wild chrysanthemum, olive, locust, dandelion, honeygrass, eucalyptus, cinnamon, cloves, orange peel, and citronella. In experiment 2, based on the results of experiment 1, a plant extract with good antimicrobial effect was selected and co-cultured with S.E and plant extract concentrations of 0.5, 1 and 2 × MIC respectively, and then the morphology and ultrastructure changes in S.E. was observed by scanning electron microscopy. The differences among the different groups were analyzed by one-way ANOVA followed by Duncan's multiple range tests using SAS software. P-values < 0.05 were considered statistically significant. Result shown that, clove extract had the best antimicrobial activity against S.E. among the 10 plant extracts, with a lowest MIC of 0.7813 mg/mL, and the largest inhibition zone of 20 mm (P < 0.05). According to the result of scanning electron microscopy, and the morphology of S.E were significantly changed supplemented with 0.5 × and 1 × MIC concentration of clove extract. The S.E treated with clove extract at the 2 × MIC concentration for 24h exhibited several changes, including cell wall fragmentation and cell cellular lysis (P < 0.05). In conclusion, the results suggest that clove extracts have active against S.E. among the 10 plant extracts in vitro, it may provide reference value for substitution of antibiotics in poultry production.

Key Words: *Salmonella enteritidis*, plant extract, minimal inhibitory concentration, scanning electron microscopy

89 Effect of long-term cyclic heat stress on turkey production and stress parameters. Benjamin N. Alig^{*1}, Dellila Hodgson¹, Jesse Grimes¹, Prafulla Regmi²; ¹*Prestage Department of Poultry Science, North Carolina State University, Wake Forest, North Carolina, United States,* ²*Poultry Science, University of Georgia, Athens, Georgia, United States.*

Modern turkey toms are often heavy birds that have been

selected for high body weight (BW) gain and low feed conversion ratios (FCR). As a result, toms can generate a considerable amount of metabolic heat. Since most of the top turkey producing states are in parts of the US that have particularly hot summers, turkeys are prone to the negative effects of heat stress in these regions. Due to these concerns, it is important to understand how cyclical, long term heat stress effects the performance of modern meat turkeys. One hundred and twenty Nicholas Select male turkey poults were brooded in battery style cages. At 4 wk the turkeys were transferred to 8 totally enclosed grow rooms with 15 turkeys/room and randomly assigned to 2 treatments. Each room contained a propane heater and ventilation system with inlets and one exhaust fan. From 4 to 5 wk the temperature in every room was raised to 90F for 8 hr between 8am and 4pm every day and then lowered to ambient temperature (between 65-75F) after 4pm. After 8 wk the heat treatment was ended in half of the rooms and these rooms were left at ambient temperature for the remainder of the study while the other half of the turkeys remained exposed to heat stressed until 18 wk. Production parameters (feed consumption and individual BW) were recorded and blood samples were drawn biweekly. At 18 wk, 2 toms/replicate were processed and carcass yield data were collected. Statistical analysis was performed using JMP Pro 14 and post hoc analysis was done utilizing the student's t-test. A p-value of $p \leq 0.05$ was considered significant while a p-value of $p \leq 0.1$ was considered trending. Non-heat stressed toms were heavier (17.95 kg) than heat stressed toms (16.96 kg) at 18 wk ($P < 0.05$). No difference was found in feed disappearance or FCR. Based on processing data, heat stressed turkeys had larger legs than non-stressed turkeys by 0.66% ($p < 0.05$). Furthermore, heat stressed turkeys had heavier wings, lighter thighs and lighter breast muscles by 0.66%, 0.38% and 1.09% respectively ($p < 0.1$). Heat stressed turkeys tended to have smaller testes by 0.05% (~ 9.37 g; $P < 0.1$). Plasma corticosterone concentration was higher in heat stressed than non-heat stressed birds at 6 wk ($p < 0.05$). In conclusion, based on this study heat stress at 90F could affect commercial tom turkey performance and carcass yield. Further research on physiological and behavioral parameters could provide greater insight into the mechanisms that help turkeys cope with chronic heat stress.

Key Words: Turkey, Heat Stress, Performance, Carcass Yield, Organ Weight

90 Heat stress and feed restriction distinctly affect carcass and body parts yield in broiler chickens. Nima Emami^{*1}, Elizabeth S. Greene¹, Barbara Mallmann², Estelle Devillard², Sami Dridi¹; ¹Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, ²Adisseo, Commeny, France.

Global warming threatens all kinds of life on earth, and increased frequency and severity of heat waves are predicted over the next decades. Broiler chickens are particularly susceptible to heat stress (HS) due to their

unique physiology and high metabolic rates, and negative effects of HS on performance are attributed to lower feed intake (FI). This study was conducted to distinguish between the effects of HS and FI depression on broiler chicken's performance, and carcass and body parts yield. In total, 720 day-old male Cobb 500 broiler chicks were randomly allocated to three treatments with 8 replicates of 28 birds/pen including: control (CTL): birds raised under normal temperature (23 °C) from d 29-42 and had free access to the diet; 2) heat stress (HS): birds exposed to high temperatures (35 °C) from d 29-42 and had free access to the diet; 3) pair-fed (PF): birds raised under the same condition as CTL group, but fed the same amount of feed as HS group from d 29-42. All birds were fed a corn-soybean meal basal diet in the form of crumble for the starter (d 0-14), or pellet for the grower (d 15-28), and finisher (d 29-42) phase. Feed and water consumption were measured daily and body weight measured weekly. On d42 gut integrity measured using FITC-d in 2 birds/pen. On d42, 15 birds/pen were processed and carcass parts were weighed. Core body temperature from 8 birds/treatment were recorded from d 29-42 using thermologgers. Data were analyzed using JMP (Pro15) with significance between treatments identified by LSD. Mortality was similar in all the treatments during the starter, grower, finisher, and overall experimental period. Core body temperature was increased $\sim 1^\circ\text{C}$ during the heat stress period in the HS group, as compared to the CTL and PF birds. Body weight (BW) and relative carcass yield was significantly higher in CTL compared to HS and PF birds on d 42. Compared to HS, PF birds had significantly higher BW and lower relative carcass yield. Birds in the HS group has higher FITC-d in the plasma compared to CTL group. Body parts yield data (relative to hot carcass weight) showed significantly higher breast yield for CTL compared to HS. In addition, breast yield was significantly higher in PF compared to HS. In contrast, leg quarters and wings yield were significantly higher for HS compared to CTL and PF. Cumulatively, these data indicate distinct effects of FI and HS on broiler chicken's performance, body parts yield and gut integrity. Thus, the negative effects of HS on broiler chicken's performance is not limited to the lower FI during the HS, and underlying mechanisms should be further investigated.

Key Words: Heat stress, broiler chicken, mortality, body weight, body parts yield

91 Biosecurity practices in relation to on-farm mortality and disease in turkey flocks. Nienke van Staaveren^{*1}, Emily M. Leishman¹, Ben J. Wood^{1, 2, 3}, Alexandra Harlander¹, Christine F. Baes^{1, 4}; ¹Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, ²Hybrid Turkeys, Kitchener, Ontario, Canada, ³School of Veterinary Science, University of Queensland, Gatton, Queensland, Australia, ⁴Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland.

Turkey farmers use biosecurity and health management

practices to limit production losses; however, it is unclear how these practices relate to disease and mortality in turkeys. As part of a larger cross-sectional survey of Canadian turkey farmers, farmers were asked to describe their farm including biosecurity and barn hygiene practices, health management, cumulative flock mortality, and disease presence in the flock. Linear (mortality, %) and logistic (disease, yes/no) regression models were used; associations between farm characteristics, biosecurity, barn hygiene, and health management practices with either mortality rate (72 responses) or disease presence in the flock (67 responses) were analysed. Explanatory variables in both models included farmer experience, flock certification, vaccination, biosecurity and barn hygiene indexes, number of age groups on farm, presence of other poultry and animal species, and use of 'hospital/sick' pens, while adjusting for flock sex and age. Indexes of biosecurity and barn hygiene practices were used as explanatory variables to address if the practices used by farmers influenced disease or mortality outcomes. Separate models were used to analyse the effect of specific biosecurity and barn hygiene practices. Approximately 30% of farmers reported the presence of disease in their flock and the average cumulative mortality was $4.1 \pm 2.9\%$ (IQR: 2.1 – 5.4%). Preliminary results showed a tendency for higher odds of disease when turkeys were kept on a multi-species farm (OR = 7.2, 95% CI = 1.1-47.8, P = 0.04). Farmers who used 'hospital/sick' pens tended to have a higher cumulative mortality in their flocks ($5.2 \pm 1.4\%$) compared to those who did not ($3.5 \pm 0.9\%$, P = 0.08). Higher mortality was found in flocks where dry cleaning (i.e., removal of litter, organic matter, dust) was used ($3.3 \pm 0.4\%$ vs $2.3 \pm 0.4\%$, P = 0.02), while mortality tended to be lower in flocks where fumigation ($2.3 \pm 0.4\%$ vs $3.2 \pm 0.3\%$, P = 0.09) was used. However, results should be interpreted with caution, as the differences in mortality were relatively low. Human-related biosecurity practices (e.g., handwashing, footwear, clothing etc.) were not associated with turkey disease or cumulative mortality in this study, although these practices are widely considered biosecurity standards. These results provide first insight into how farm characteristics, biosecurity, barn hygiene, and health management practices are associated with disease and mortality in Canadian turkey flocks, however, further investigation to elucidate causal relationships is required.

Key Words: Turkey, Health, Biosecurity, Disease, Mortality

92 Effect of housing system environment on brown egg layer quality (internal and external). Ramon D. Malheiros*, Kenneth Anderson; *Prestage Department of Poultry Science, NC State University, Raleigh, North Carolina, United States.*

The housing system (HSy) is an external factor that influences both the performance of hens and the egg quality characteristics. Conventional cages have been banned in the EU since 2012, and the housing of laying hens is only permitted in alternative systems enriched cages, such as

cage free housings, aviaries or free range, these systems which are believed to improve the welfare of the hens. The objective of the study was to evaluate the quality of eggs from brown laying hen strains based on HSy. The quality analysis was made on eggs from commercial brown strains used in the US and EU market. Five housing systems were: Cage Free (CF), Enrichable Cage (EC), Enriched Cage (ErC), Conventional (CvC), and Free Range (FR). Eggs were evaluated of three hen ages 25-28 wks., 33-36 wks., and 49-52 wks. In each period 744 eggs, were randomly chosen from each HSy, equally representing each strain. The HSy were evaluated based on the proportion of the house size (CF 120, EC 192, ErC 192, CvC 192, and FR 48 eggs resp.) contributing to the total sample. In each hen age all eggs were evaluated for a set of physical characteristics: shell color, egg weight, albumen height, Haugh unit, yolk color (TSS, York, England), shell strength and elasticity, and vitellin membrane strength and elasticity (Texture Analyzer, Surry, England). Statistical analysis was done using JMP 15.2, and significant means differences between hen age and HSy were determined using Tukey's HSD. The differences between the three ages were highly significant (P < .0001) in all characteristic evaluated, which is expected as hens age. Shell color was darker brown in EC, than the others HSy (P < .0001), and in the FR system eggs were heavier (P < .0001) than other HSy, while the Haugh unit was lower in CF, (P < .0001). The darker yolk color was showed in FR HSy (P < .0001). Interestingly the shell quality characteristics showed that eggs from CF and FR HSy, have greater shell strength and elasticity (P < .0001), followed by EC, ErC, and CvC HSy showed a very similar shell quality, respectively. The vitellin membrane strength and elasticity were not affected by the HSy, with P values of 0.1182, and 0.3264, respectively. In conclusion, the housing systems of CF and FR, appear to improve the external and internal egg quality. This may be related to better interplay of the laying hens within the environment, with the ability to express an expanded behavior profile including walking, nesting, coprophagy, and access to pastures.

Key Words: Layer Hens, Housing systems, Egg Quality

93 Enumeration of naturally occurring microorganisms in the intestinal tract and yolk sac of day-old chicks from commercial hatcheries. Karely Cantu*, Craig Coufal; *Poultry Science, Texas A&M University, College Station, Texas, United States.*

Previously published experiments have demonstrated that eggs inoculated with pathogens prior to hatching can result in day-old chicks being contaminated with those same pathogens. Other studies have investigated the extent of naturally occurring *Salmonella* contamination in day-old chicks, but less focus has been given to other potentially pathogenic species of microorganisms. Such microorganisms may inhabit the chick's gastrointestinal tract microbiota and may lead to morbidity or early mortality during grow-out. With many poultry companies implementing antibiotic-free production, chick

contamination from the hatchery is of great concern. The objective of this study was to determine the extent of naturally occurring microbial contamination in the intestinal tract and yolk sac of day-old chicks obtained from commercial hatcheries. Ten chicks were obtained from 6 different hatcheries, representing primary breeder and integrator operations, and transported to the lab for microbial evaluation. Following chick euthanasia, aseptic techniques were used to remove the intestinal tract (duodenum to colon) and yolk sac from each chick and individually placed into separate sterile bags. Sterile PBS was added to each bag at four times the sample weight to achieve an initial 1:5 dilution. The contents of the bags were homogenized by manual massage. Ten-fold serial dilutions were performed, and aliquots were plated onto various aerobic and anaerobic media to enumerate total aerobic microorganisms (APC), *Staphylococcus* spp., *Escherichia coli*, total coliforms, fungi, *Clostridium* spp., and *C. perfringens*. For all hatcheries, the average chick intestinal tract counts for APC, *Staphylococcus* spp., *E. coli*, total coliforms, fungi, *Clostridium* spp., and *C. perfringens* counts were 7.57, 5.89, 6.10, 6.95, 5.49, 7.57, and 2.06 log₁₀cfu/g of intestine, respectively. The average yolk sac counts for all hatcheries were 3.15, 1.02, 2.31, 2.92, 3.33, 3.46, 1.52 log₁₀cfu/g for APC, *Staphylococcus* spp., *E. coli*, total coliforms, fungi, *Clostridium* spp., and *C. perfringens*, respectively. The frequency of detectable counts for APC, *Staphylococcus* spp., *E. coli*, total coliforms, fungi, and *Clostridium* spp. for all chick intestinal samples was nearly 100%, whereas the frequency of detectable counts for yolk sacs was significantly lower. The frequency of *C. perfringens* detectable counts in chick intestines was 36.6%, whereas *C. perfringens* was detectable in only 6.6% of the yolk sac samples. The data obtained in this study demonstrated that the gastrointestinal tract of day-old chicks from some commercial hatcheries may have high counts of potential pathogens.

Key Words: chick, intestine, yolk sac, contamination, bacteria

94 Microbiota composition of the ileum and ceca of colony-enriched and cage-free egg layer production systems. Dana K. Dittoe*¹, Anita Menconi², Steven C. Ricke¹; ¹*Meat Science and Animal Biologics Discovery, Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, Wisconsin, United States*, ²*Evonik Corporation, Kennesaw, Georgia, United States*.

With the continuing increase in animal welfare standards, many egg producers have shifted their production systems to those of colony-enriched or cage-free production systems. However, there is still much debate on the effect the systems have on the health and gastrointestinal (GIT) microbial ecology; thus, research investigating the impact these systems have on the GIT microbiota must be explored. Therefore, the objective was to compare the microbiota populations of layers reared in enriched and cage-free production systems during peak production. At a

commercial egg production facility, layers were reared in either enriched cages (710 cm²/bird) equipped with a nest curtain and perch bar (Hy-line W-36), or aviaries (Hy-line Brown) within the same housing facility. A total of 30 layers (38 wk; n = 15; N = 30, k = 2) were humanely euthanized. The distal ileum and ceca were aseptically collected, and the genomic DNA of the contents were extracted. The 16S rDNA targeting the V4 region was sequenced on an Illumina MiSeq. Microbiota data were filtered and aligned using the QIIME 2-2020.11 pipeline, with data considered significant at P ≤ 0.05 for main effects and Q ≤ 0.05 for pairwise differences. There were more observed features (Observed Operational Taxonomic Units) and a greater diversity (Shannon Index) of the microbiota within the ceca of the layers reared in the enriched system (P < 0.05; Q < 0.05), while there were differences between the observed features or diversity (Shannon Index) of the two systems on the ileum microbiota (P > 0.05; Q > 0.05). Also, the ileum and ceca of the cage and cage-free layers had different microbial abundances and phylogenies, as demonstrated by Bray Curtis and Weighted Unifrac (P < 0.05; Q < 0.05). Using ANCOM, the analysis of the composition of microbiomes, *Canidatus* *Arthromitus* (W = 427), commonly associated with arthropods such as beetles and termites, was more abundant in the ileum of caged layers than among cage-free layers. As well, ANCOM revealed that caged birds had a higher relative abundance of *Akkermansiaceae* (W = 0), a potential indicator of a healthy GIT, and cage-free layers having a higher abundance of *Methanobacteriaceae* (W = 15), an archaeal methanogen capable of methane production. In conclusion, there were apparent differences in the microbiota of the ileum and ceca of commercial layers reared in enriched and cage-free systems, with layers reared in enriched cages having a richer and more diverse microbiota. These results potentially indicate the potential improvement of the microbiota of the ileum and ceca of layers reared in enriched systems to that of cage-free egg-production systems.

Key Words: poultry, 16S rDNA, cage-free, enriched cages, microbiota composition

95 Evaluating the effects of stocking density on the health and behaviour of turkey hens to 11 weeks of age. Sameeha Jhetam*^{GS 1}, Karen Schwan-Lardner², Kailyn Beaulac¹; ¹*Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada*, ²*Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada*.

Stocking density (SD) is a key factor that impacts the health and welfare of birds by possibly affecting chronic stress and behaviour. Few studies exist that assess turkey health and welfare in relation to SD, with greater focus on toms compared to hens. This study (two trials) examined the effects of SD on turkey hen health and wellbeing to 11 wk of age. Nicholas Select hens (n=3550 poults/trial) were randomly placed in one of four final estimated SD treatments (trt) of 30, 40, 50, or 60 kg/m² with four

replications per trt. Birds were housed in open rooms (67.5m²) and feeder and drinker space were equalized on a per bird basis. Air quality (CO₂ and ammonia) were tested daily, and ventilation was adjusted to equalize air quality across all trt. Heterophil/lymphocyte (H/L) ratios were evaluated at 3, 5, 8, and 11 wk of age as a measure of chronic stress (20 birds/replicate). Behaviour was recorded and scan sampled (field of view observations) at 8 and 11 wk of age. Litter samples were collected at wk 8 and 11 of trial 1 only. Samples were dried and weighed to calculate average litter moisture. Data were analyzed using regression analysis in SAS 9.4 (Proc Reg and Proc RSReg; SD as independent variable). Differences were considered significant when $P < 0.05$. All values reported are in ascending order of SD trt 30, 40, 50, and 60 kg/m². At wk 3, no differences were noted for H/L ratios. At wk 5, H/L ratios linearly increased as SD increased (0.76, 0.85, 0.88, 0.89; $P < 0.01$). At 8 wk of age, H/L ratios were highest in the 40 kg/m² trt birds (quadratic; 1.09, 1.4, 0.83, 0.89; $P = 0.03$). At wk 11, H/L ratios increased with increasing SD (quadratic; 0.87, 0.96, 1.13, 1.06; $P < 0.01$). Behaviour was impacted at 8 wk as the percent of birds feeding ($P = 0.04$), resting ($P < 0.01$), and total disturbances ($P = 0.05$) linearly increased with increasing SD. Bird activity linearly decreased as more birds were standing ($P = 0.02$), walking ($P < 0.01$), and litter pecking ($P = 0.03$) in 30 kg/m² trt. Aggressive behaviour (fighting plus aggressive pecking) was highest in the low SD trt (quadratic; 0.60, 0.34, 0.11, 0.19 %; $P = 0.01$) at wk 8. At wk 11, bird drinking (quadratic; $P = 0.02$), walking (linear; $P = 0.03$), and head scratching (linear; $P = 0.02$) decreased with increasing SD. There was a tendency for birds in the 30 kg/m² trt to perform more environmental pecking (quadratic; $P = 0.07$). Resting ($P < 0.01$), feather pecking ($P = 0.01$) and severe disturbances ($P = 0.02$) linearly increased as SD increased. Litter moisture was not affected by SD. The results indicate that bird health and welfare is negatively affected by higher SD through increased chronic stress and altered behaviour such as decreased mobility, however, low SD may increase aggressive acts.

Key Words: stress, aggression, mobility behaviours, litter moisture, heterophil/lymphocyte ratio

96 In pursuit of a better broiler: Tibia morphology, breaking strength, and ash content in conventional and slower-growing strains of broiler chickens. Midian Nascimento dos Santos*^{GS 1}, Tina Widowski¹, Elijah Kiarie¹, Michelle Guerin², Stephanie Torrey¹; ¹*Animal Biosciences, University of Guelph, Guelph, Ontario, Canada*, ²*Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada*.

The rapid growth rate observed in fast-growing strains of broiler chickens has been linked to bone disorders that can cause lameness. Thus, the use of slower-growing (SG) strains has been suggested as an alternative for improved bone quality. To determine the impact of growth rate on bone traits, a total of 7,216 mixed-sex birds representing 14 strains were investigated, including 2 conventional (CONV)

strains (strains B and C: $ADG_{0-48} > 60$ g/d), and 12 SG strains categorized in 3 groups based on their growth rate. The SG strains were classified as FAST (strains F, G, I, and M; $ADG_{0-62} = 53 - 55$ g/d), MOD (strains E, H, O, and S; $ADG_{0-62} = 50 - 51$ g/d), and SLOW (strains D, J, K, and N; $ADG_{0-62} < 50$ g/d). The birds were sexed at hatch and placed in 164 pens (44 birds/pen, 30 kg/m²). Each strain was represented in 4 pens over 2-3 production cycles, totaling 8-12 pens per strain in a randomized incomplete block design. From each pen, 4 birds (2 males and 2 females) were individually weighed and euthanized at 2 target weights (TWs) according to the time they reached 2.1 kg (TW1: 34 d for CONV and 48 d for SG strains) and 3.2 kg (TW2: 48 d for CONV and 62 d for SG strains). Tibia samples were dissected, length and diameter recorded. Left tibia was used for bone-breaking strength at both TWs and tibia ash at TW2. Data were analyzed using Proc Glimmix in SAS 9.4. For all of the models, category, strain, sex, TW, and the interactions were fixed effects, and block and production cycle random effects. For comparisons at the same age (48 d), category, strain, and sex were fixed effects, and the interactions were kept if significant. At TW1, CONV birds' tibia were narrowest and shortest ($P < 0.001$), yet had similar tibia breaking strength (TBS) compared to the other categories ($P > 0.69$). At TW2, categories ($P > 0.50$) had no effect on tibia diameter, yet CONV birds had the shortest tibia ($P < 0.001$). The CONV birds had greater TBS:BW ratio than FAST and MOD birds at TWs 1 and 2 ($P < 0.039$). The CONV birds had the lightest tibia ash weight relative to BW ($P < 0.001$) and lighter absolute tibia ash weight than FAST and MOD birds ($P < 0.009$), yet similar ash content to the other categories ($P > 0.22$). At the same age, CONV birds had the greatest absolute TBS ($P < 0.003$), yet lower TBS:BW ratio than SLOW birds ($P < 0.001$). Tibiae from CONV birds were longer than MOD and SLOW birds, and thicker in diameter than the other categories, yet CONV birds had the lowest relative dimensions ($P < 0.001$) at a similar age (48 d). In conclusion, both genetic selection for growth and age affected bone traits. Future studies should investigate if the differences observed impact walking ability and welfare of broiler chickens.

Key Words: Chickens, Slow-growth, Bone health, Growth rate, Lameness

97 Impact of Orego-Stem® on feed, forage, weed seed, insect and other crop contents in a range setting. Kari L. Harding*^{GS 1}, Benjamin N. Alig¹, Rebecca Wysocky¹, Kenneth E. Anderson¹, Ramon D. Malheiros¹, Wendy Wakeman²; ¹*Prestage Department of Poultry Science, North Carolina State University, Wake Forest, North Carolina, United States*, ²*Anpario plc, Worksop, Nottinghamshire, United Kingdom*.

Due to consumer demand, the egg industry is moving away from caged bird systems to cage-free and free range system. Many products are being used to improve gut health and effectively improve productivity in layers. Oregano (*Origanum vulgare*) essential oil (OEO) contains over 100

compounds with active properties. Four key compounds are carvacrol, thymol, ρ -cymene, and γ -terpinene that have been shown to have antibacterial, anti-inflammatory, and antioxidant properties. OEO has been shown to be beneficial for gut health, and subsequent growth and production performance in poultry. Currently, there is limited knowledge on how these oregano compounds may interact with forage nutrients consumed by the birds from the range. Three dietary treatments were utilized in this study, a control through rearing and lay (CC), a Control through rearing and Oregano-Stem through lay (CO), and Oregano-Stim through rearing and lay (OO). Pullets were reared in a range house on litter from 0-6 wks, then slats until 12 wks, after which were then allowed on range. Crop contents were collected at the start of the trial, 17 wks, and trialend, 41 wks. The components examined in this study were feed, forage, weed seeds, insects and other (i.e. rocks, feathers, eggshells) consumed as supplemented feed or foraged consumables. Crop contents were excised into Nalgene cups, placed in cooler, and brought back to the lab and dried at 50 C for 72hr. Samples were broken apart and components visually separated using a dissection scope for analysis in the lab. Full crop contents were weighed, then each component was separated under the microscope and weighed individually to determine intake. The component intake of each was then calculated on a dry matter percentage basis. Data was analyzed using JMP Pro 15 using the least squares method and post hoc analysis was done utilizing the student's t-test. A p-value of $p \leq 0.05$ was considered significant. Crops that were sampled at wk 17, had consumed significantly more weed seeds compared to those sampled at wk 41. Crop contents from wk 17 showed to have eaten significantly more insects than the older birds. This could be due to the seasonal placement of the birds in the springtime, when more weed seeds and insects are present. This could also be a result of the birds eating all the weed seeds and most of the insects when they were first allowed on the range, and the prevalence of weed seeds and insects were diminished when sampled the second time. The treatments, however, did not have an impact on any of the components consumed. In conclusion, birds that are placed on range pastures do consume other nutrients aside from their given feed and intake appears consistent across differing diets.

Key Words: Laying hens, crop contents, free range, oregano essential oil

98 Assessment of Pulsed Alternating Wavelength System (PAWS) on musculoskeletal quality of market-age Pekin ducks. Brittney Emmert*^{GS 1}, Jason Suntych², Daren Suntych², Marcus Reinhardt², Darrin M. Karcher¹, Gregory S. Fraley¹; ¹*Animal Sciences, Purdue University, West Lafayette, Indiana, United States*, ²*Xiant Technologies, Inc., Greeley, Colorado, United States*.

The use of artificial lighting in poultry production is a necessity to mimic the long day lengths needed for optimal growth and production in enclosed housing systems. Even

though lighting is critical for proper development of poultry, there is minimal research on the effects of different lighting technologies on growth and production, especially in ducks. Pulsed Alternating Wavelength System (PAWS) is a novel LED technology that is able to provide multiple wavelengths of light to growing animals and may be beneficial for their growth and welfare as opposed to monochromatic light that has been shown to produce undesirable effects. As with all meat birds, the fast growth rate of ducks can lead to skeletal abnormalities and lameness. The purpose of this study was to ensure PAWS does not cause detriment to the skeletal system while enhancing the growth rate of ducks. Ducks were raised on one of five lighting recipes (40 ducks per treatment): industry control (fluorescent light 18L:6D), PAWS 1 (Grow4 20%, 24L), PAWS 2 (Grow5 20%, 24L), PAWS 3 (Grow5 20%, 17L:7D), and PAWS 4 (Grow5 20%, 18L:6D). At 30 days of age, five ducks per treatment were sacrificed and the keel with breast muscle, and both wings and legs were collected. The humerus, femur, and tibia were separated, with muscle attached, from the wing or leg, respectively. Weight of the muscle surrounding each bone (keel, humerus, tibia, and femur) was calculated by weighing each piece intact (bone with muscle), cleaning all tissue from each bone, and subtracting the cleaned bone weight from the intact weight. Each tibia and humerus were measured for length and width, then the right bones were subjected to a 3-point bending test for breaking strength. Subsequently, the right tibia and humerus were ashed to determine bone mineral content. All data were analyzed using one-way ANOVA and significance was detected at $P < 0.05$. Average muscle weight of the femurs from PAWS 4 was greater than PAWS 2 (60.13 g vs. 47.07 g; $P = 0.04$). PAWS 4 had longer humeri than PAWS 2 (110.43 mm vs. 103.65 mm; $P < 0.01$) and PAWS 3 had wider femurs than the control (7.68 mm vs. 7.19 mm; $P = 0.04$). There were no differences between treatments for all other muscle weights and bone measurements ($P > 0.05$). Remaining bone quality parameters (breaking strength and bone mineral content) of both humeri and tibiae were similar across treatments ($P > 0.05$). The novel PAWS lighting does not result in an inferior skeleton compared to conventional lighting and may improve femur and humerus size. These results are encouraging and further studies on the potential benefits of PAWS on duck production and welfare measures are warranted.

Key Words: duck, lighting, bone, muscle, measurements

99 Increasing welfare by placing circulation fans in commercial broiler houses. Zoie N. McMillian*^{GS 1}, Jonathan Moyle², Shawna L. Weimer¹; ¹*Animal and Avian Sciences, University of Maryland, College Park, Maryland, United States*, ²*Extension, University of Maryland, Salisbury, Maryland, United States*.

Additional air movement in commercial houses could positively affect the welfare of broilers by reducing footpad dermatitis prevalence and stabilizing environmental

conditions. We hypothesized that broilers raised in houses with circulation fans would have higher welfare than without. This study was conducted in 4 commercial organic broiler houses on a farm in eastern Maryland. Two houses had high-capacity circulation fans (House 1 and 2) and 2 did not (House 3 and 4). Each house was divided into 4 quadrants for welfare assessments at 3 and 6 weeks. The cool cells were located in quadrant 4, tunnel fans at the far end of quadrant 1, and brooding took place in quadrants 1 and 2 until the chicks were 10 days of age. Prior to chick placement, 2 data loggers were set to record ambient temperature (°F) and relative humidity (%) every 15 minutes throughout each house. Litter samples were taken from each house at week 0, 3, and 6 to determine litter moisture content (%). During each welfare assessment, 80 birds within 2 locations of each quadrant (N=10 birds/location) were weighed and scored for footpad dermatitis, hock burn, and feather cleanliness. Ambient volume (dB) measurements were taken at every welfare assessment location. The transect method was also performed in all 4 houses at the time of the week 6 welfare assessments. Body weight, transect, and environmental data was analyzed with ANOVA. Welfare data was analyzed with chi-square test. House 4, one of the hotter houses, had the highest number of observed small birds at week 6, compared to the other 3 houses ($P \leq 0.04$). House 4 also had the highest litter moisture content (28%) at week 6, compared to the other 3 houses ($P < 0.0001$). Environmental data indicated that the volume (dB) in Houses 3 and 4 was quieter, by about 6.6dB than the volume in Houses 1 and 2 at the time of the week 3 assessment ($P < 0.0001$). As expected, footpad dermatitis prevalence increased with bird age. Although they can increase noise levels, high-capacity circulation fans could potentially be a cost-effective way for producers to increase broiler welfare. More research is needed to determine the effects of circulation fans and adequately evaluate their benefits to broiler management and welfare.

Key Words: Welfare, Broilers, Footpad dermatitis, Circulation fans, Litter moisture

100 Robotic poultry carcass picker. Jennifer Koch^{UG*}, Joseph Ishak, Sam Boone, EmmaLi Clark; *Biological and Agriculture Engineering, North Carolina State University, Clayton, North Carolina, United States.*

This project includes the design, construction, and testing of a robotic turkey carcass collector made by modifying a commercially available remote-controlled robot. This system was created in response to the labor shortages, the increase in average age of poultry producers, and the drudgery of tasks in the poultry industry with a focus on turkey facilities. There is an opportunity within the recent growth of robotics to modify a remote-controlled robot to collect poultry carcasses from the poultry house and inspect both flock welfare and equipment functionality. Ideally, this system would enable a poultry producer to sit in their office and control the different components of the robot remotely.

The base robot provided is controlled by a 2.4 GHz controller/receiver combo, around which the development of the other components was done. To detect the turkey carcasses in the farm, a monitoring system was installed on the base robot consisting of a total of two wide-angle cameras mounted on the front and the back, that can be accessed remotely by the operator. The operator can switch between the two views using the main controller. To lift the carcasses a front fork was designed, built, and connected to the base robot. An electrical linear actuator, operated using the main controller, moved the fork up and down to reach the desired position. On top of the robot, a revolving plastic dump bed is used to contain the carcasses until it is unloaded by the operator. Testing was completed using a 50lb. sandbag in a mulched flowerbed to simulate a poultry house. The results include the successful lifting of the test weight and the remote navigation of the robot using the camera system. In conclusion, a robotic system such as the one tested could be useful to poultry growers to collect the carcasses. Future research is needed to refine the lifting and transportation of the carcasses as well as the range of remote controller and camera systems. The broader impacts of the Robotic Poultry Carcass Picker include AgrAbility and biosecurity.

Key Words: Carcass Collection, Robotic Poultry Management, Precision Livestock, AgrAbility, Biosecurity

101 Comparison of a standard male diet to an organic selenium or docosahexaenoic acid (DHA) supplemented male diet on rooster semen quality and reproductive performance. Kelly M. Sweeney^{GS 1}, Jeanna Wilson¹, Luis P. Avila²; ¹*Poultry Science, University of Georgia, Athens, Georgia, Georgia,* ²*Poultry Science, University 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, or that hen diets have too high a level of calcium or protein for roosters. In this study, a basal diet with 2720kcal/kg energy, 12% crude protein, 0.74% calcium, and 0.15ppm of sodium selenite was mixed (Aviagen, 2016) specific to the roosters needs. The basal was split into three diets with the male control (MC) diet containing a total of 0.30ppm of sodium selenite, male organic (MO) diet having 0.15ppm of organic selenium, and male DHA (MD) diet containing 0.28% DHA and 0.30ppm of sodium selenite. Docosahexaenoic acid (DHA) is an omega-3 fatty acid that is proven to improve soft tissue development like brain tissue and testes and other omega-3 fatty acids have been proven to improve semen quality (Hudson, 2003). Selenium is a trace mineral that is important for testosterone metabolism and sperm motility. The objective of this study was focused on evaluating the dietary inclusion of organic selenium or DHA as separate strategies would improve rooster semen production and quality when compared to a traditional male diet. All treatment groups were fed equal amounts of feed from

16wks to 45wks. A total of 60 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 5wks from 25 to 45wks. Individually caged Ross 708 hens were artificial inseminated with 0.05 mL pooled and diluted semen from a replicate group of males. Semen was diluted with Avian Buffer to 7.5×10^7 sperm. Eggs were collected for 14 days' post-insemination and incubated for a fertility analysis. Data were analyzed by SAS 9.4 SLICE and means separated by LSD. Significance level was $P \leq 0.05$. None of the diets had significant influence on body weight, semen volume, or semen concentration. Sperm mobility was significantly impacted from week 34- 45wks ($P < 0.05$) with the males fed the MD and MO diet having greater sperm mobility than the mobility of the MC males. The dietary supplementations had no significant impacts on fertility through 45wks. While early in life fertility and semen concentrations are not significantly impacted, further research with older males needs to be conducted to determine if DHA or organic selenium impacts late in life fertility and semen quality.

Key Words: Rooster Diet, Semen Quality, Fertility, Selenium, Omega 3 Fatty Acid

102 Supervised machine learning models to predict body weight and feed conversion ratio of broilers reared under commercial conditions. Gustavo Quintana-Ospina*^{GS 1}, Edgar O. Oviedo-Rondon¹, Maria C. Alfaro-Wisaquillo², Luis Carlos Bernal-Arango³, Gustavo Martinez-Bernal³; ¹*Prestage Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, United States*, ²*Poultry Science, North Carolina State University, Raleigh, North Carolina, United States*, ³*Grupo BIOS Inc., Envigado, Antioquia, Colombia*.

Machine learning (ML) models are useful tools to predict final BW prior to slaughter age to make data-driven decisions. This study evaluated the performance of three supervised ML methods, multiple linear regression (MLR), random forest (RF), and neural networks (NN), in the prediction of BW and feed conversion ratio (FCR) of broilers at 35d, reared under tropical commercial conditions with control feeding. Performance records until 21d from 1,188 male and 1,193 female Ross AP broiler flocks, housed in 85 farms were analyzed for a total of 84 million chicks placed between 2018 and 2020. Farm management conditions were collected using a survey. Data were divided into training and testing in 5 folds for cross-validation to test model predictability. RF and NN were trained with the *Caret* package, and MLR was fitted with the *lm* function in R software. The best *m* value and nodes number were obtained from hyperparameter tuning for RF and NN, respectively. In MLR, multicollinearity was assessed with the variance inflation factor, thus collinear and non-

significant variables were removed. The R^2 and RMSE were calculated from the 5 testing folds. Feature importance was determined by percentage increase in MSE using permutation importance in RF and Connection Weights method in NN. In MLR, variable importance was expressed as the variable sum of squares percentage from the total sum of squares. On chicken BW, RF resulted in $R^2 = 0.78$ and RMSE = 56.74, NN in $R^2 = 0.73$ and RMSE = 62, and MLR in $R^2 = 0.59$ and RMSE = 76.38. RF, NN, and MLR included within the ten first most important variables, chicken sex, and feed intake (FI) at 14d. RF and NN also had in common BW, FI, and BW gain at 21d, and downtime between flocks. Other variables displayed in the feature importance included the farm distance from hatchery, farm altitude and stocking capacity, hatchery location, and BW, BW gain, mortality, and ADG at 14d. On FCR, RF resulted in $R^2 = 0.48$ and RMSE = 0.041, NN in $R^2 = 0.45$ and RMSE = 0.042 and MLR in $R^2 = 0.18$ and RMSE = 0.052. Variable importance comparison indicated that FI and FCR at 21d were considered by all FCR models. RF which accounted for the best goodness-of-fit metrics included BW, BW gain and FCR at 14d as important variables. Farm altitude and farm distance from hatchery also presented higher percentage increase in MSE. However, the low model fit indicates that FCR 35d can be associated to nutritional and environmental variables which were not considered in this study. In conclusion, RF was the best model to predict BW two weeks prior to slaughter age. Performance parameters at 21d, sex, farm distance from hatchery, and farm altitude demonstrated to play an important role in the broiler BW at 35d.

Key Words: Machine learning, random forests, neural networks, broilers, body weight.

103 Verification of RFID for tracking of poultry in multi-tiered aviary systems. Torey J. Fischer*^{UG1}, Cara Robison¹, Darrin M. Karcher², Prafulla Regmi³; ¹*Department of Animal Science, Michigan State University, East Lansing, Michigan, United States*, ²*Department of Animal Sciences, Purdue University, West Lafayette, Indiana, United States*, ³*Department of Poultry Science, University of Georgia, Athens, Georgia, United States*.

Radio frequency identification (RFID) has been used extensively for inventory tracking and for general traceability in warehouse management and more recently in segments of animal agriculture. However, use of RFID has not been explored extensively to track the movement of birds in a multi-tiered aviary system. Verification of these systems is especially relevant now to monitor space use and in improving cage design to ensure bird welfare and production efficiency as the egg industry makes the switch to cage-free housing. The purpose of this project was to validate an RFID system to ensure accuracy in data collection for tracking and monitoring hens. Hens were housed in 4 aviary rooms, each with 4 sections, at a minimum space allocation of 929 cm² per bird. One section in each room was selected for RFID installation. A traveling

wave RFID antenna was installed on each tier (bottom, middle and top) of the aviary and digital video cameras were installed to record and confirm the bird location with the RFID tag. In that section, 40 out of 144 total hens were leg banded with an RFID tag. Video was recorded for three consecutive days in each section. Additionally, 12 focal birds out of the 40 in each room were tagged with uniquely colored plastic leg bands to allow for identification on video. Video data was correlated to RFID data to determine if the antennas were accurately registering the bird's location. Times when birds appeared near antennas on the video were marked and then cross-referenced with times when birds were recorded near the same antenna by the RFID reader. For each level of each room about 30 hits were confirmed on the video data, giving a total of 400 hits validated. There was an issue with one of the cameras part way through recording, resulting in missing validation from the top level of one room. Data from those confirmations were analyzed using Proc Freq procedure in SAS 9.4. On average, 87.3% of the birds were confirmed; however, the top tier had the highest confirmation rate at 93.5%. It was concluded that the RFID system was reading accurately. In instances where birds were not confirmed it was typically an issue visualizing the bird on the video due to lighting, position in the cage, or too many birds in a frame. In the future, this type of technology could be utilized to give producers critical information about how birds are using these types of systems. This information is helpful in improving cage design to be the most functional for how the bird are using the system. Additionally, this could impact genetic companies by identifying phenotypes of hens that best use the systems to improve well-being and to improve production through the reduction of mislaid eggs.

Key Words: laying hens, multi-tiered aviary systems, RFID, video

104 Effects of growth dynamics and feed intake on the reproductive performance of broiler breeders. Maria C. Alfaro-Wisaquillo*^{GS 1}, Gustavo Quintana-Ospina¹, Edgar O. Oviedo-Rondon¹, Luis Carlos Bernal-Arango², Gustavo Martinez-Bernal²; ¹*Poultry Science, NC State University, Raleigh, North Carolina, United States*, ²*Grupo BIOS Inc., Envigado, Antioquia, Colombia*.

Growth patterns and feed intake of pullets influence the reproductive performance of broiler breeders. The adequate balance among growing parameters has not been well explored and data analytics of commercial flocks may reveal these relationships. This study aimed to determine the importance of growth dynamics, cumulative feed intake (CFI), and flock uniformity (FU) in the number of hatchable eggs, chicks per hen housed (HH) and hatchability of Ross 308 AP broiler breeders at 55 wk reared under tropical commercial conditions. Data evaluated contained growth and reproductive results of 45 broiler breeder flocks housed between 2017 and 2019 for a total of 1.4 million hens. Partition trees were used to classify breeder flocks as low (LP), and high (HP) performance based on the eggs per HH

at 55 wk. Differences between groups in other reproductive parameters were assessed by one-way ANOVA using the student's t-test for mean comparison. Decision trees were fit with BW, CFI, and FU to identify critical points during rearing that affect the reproductive performance. Significant differences between groups were detected in all parameters evaluated. Breeder flocks from the HP group laid 11.3 hatchable eggs more ($P < 0.05$) and produced 10.6 additional chicks per HH compared to the flocks in the LP group at 55 wk. From 25 to 29 wk and 31, 37 and 44 wk of age significant differences were observed in hatchability percentage. The HP group had more chicks ($P < 0.05$) with 2% higher average hatchability than the LP breeder hens during the whole cycle. Decision trees at 3 wk estimated that pullets heavier than 390 g with a CFI of 605 g resulted in 10.3 fewer hatchable eggs per HH at 55 wk ($R^2 = 0.63$), compared to pullet flocks lighter than 390 g with FU greater than 75.5%. Similarly, pullets at 4 wk with more than 512 g and CFI higher than 864 g laid 11.7 eggs less ($R^2 = 0.70$) than lighter pullets with uniformity greater than 73.5%. Pullets weighing between 686 and 697 g at 6 wk of age ($R^2 = 0.60$), laid 11 eggs more on average compared to pullets with BW greater than 716 g at the same age. Variable contribution detected BW as the most important factor during the first two and the last four wk of the rearing period, while CFI gains importance between 3 and 18 wk of age. In conclusion, data analytics tools helped determine important points during rearing that affect hatchable eggs, hatchability, and chicks per HH in broiler breeders reared under commercial conditions. Achieving target BW and FU during the early and late rearing to avoid greater feed restrictions in grower and developer phases could improve reproductive parameters in broiler breeders.

Key Words: Growth dynamics, Feed intake, Flock uniformity, broiler breeders, Data analytics

105 Effects of light intensity variation on late-lay hen performance. Maddison L. Wiersema*^{GS}, Dawn Koltes; *Department of Animal Science, Iowa State University, Ames, Iowa, United States*.

Lighting is known to be one of the major factors affecting poultry productivity and behavior. Maintaining uniformity in light intensity is crucial in maximizing production and efficiency of flocks. However, the effects of slight differences in light intensity associated with in-house variation has not been characterized in laying hens. During a late-lay hen study, visual differences were noted in light intensity. Therefore, this retrospective study was conducted to evaluate the effects of light intensity on egg quality, mortality, and production in late-lay hens (60 weeks of age). Hens were housed in 48 cages with 6 hens placed at the start of the trial per cage. Cages were located on various tiers of a four-tier system. Light intensity measurements were collected at 3 locations: bird height at feeder, bird height at the middle of the cage, and bird height at the back of the cage. Individual egg weights (g) and hen mortality were collected daily. Egg characteristics, albumen height (mm),

Haugh unit, shell thickness (mm), and shell strength (N), were collected on 3 consecutive days per week. Weekly hen-day egg production (HDEP%) was calculated. Light measurements taken at the bird height at feeder location had the largest variability, ranging from 4.98 to 46.70 lux with an average of 15.66 lux. Light intensity measurements taken at bird height at the middle of the cage ranged from 0.81 to 11.88 lux with an average of 3.15 lux. Light intensity measurements taken at bird height at the back of the cage ranged from 0.48 to 5.67 lux with an average of 2.35 lux. To determine relationships between light intensity and weekly averaged hen data, Pearson correlations were calculated in SAS. Following the 15-week study, mortality percentage was significantly correlated with light intensity at bird height at the middle ($r = 0.447$; $P = 0.001$) and back ($r = 0.435$; $P = 0.002$) of the cage. Hens housed in cages

with lower light intensity at bird height at the middle and back of cages had lower mortality than hens housed in cages with higher light intensity at the same location. A weak, but significant, correlation between light intensity and egg weight ($r = -0.273$; $P = 0.001$) and shell thickness ($r = -0.221$; $P = 0.007$) was observed during the 3-week baseline collection with higher light intensity at bird height at feeder negatively associated with egg weight and egg-shell thickness. Variations in light intensity at all 3 locations were not significantly correlated with other traits measured. In conclusion, variation in light intensity in group-housed systems may contribute to hen mortality and lead to changes in egg quality and production.

Key Words: light intensity, egg production, egg quality, late lay hens, mortality

Metabolism and Nutrition: Amino Acids

106 Insufficient dietary sulphur amino acids exacerbate oxidative stress in broilers reared in heat stress and subsequently compromise broiler meat quality. Rose Whelan*¹, Johanna Zeitz², Anne Fleischmann², Tamara Ehbrecht², Erika Most², Denise Gessner², Klaus Failing³, Dieter Luetjohann⁴, Klaus Eder²; ¹*Animal Nutrition, Evonik Operations GmbH, Birmingham, United Kingdom*, ²*Institute of Animal Nutrition and Nutrition Physiology, Justus-Liebig-University Giessen, Giessen, Germany*, ³*Unit of Biomathematics and Data Processing, Faculty of Veterinary Medicine, Justus-Liebig-University Giessen, Giessen, Germany*, ⁴*Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, Bonn, Germany*.

Rearing broiler chickens in high ambient temperatures creates oxidative stress which may in turn increase cholesterol oxidation products (COPs) in meat which have been linked with cardiovascular disease and cancer in humans. Glutathione is the most abundant intracellular antioxidant and is synthesized from the essential sulphur amino acids, methionine and cysteine (M+C). This study investigated whether increased dietary M+C could inhibit oxidative stress and prevent meat quality issues in broilers raised at high temperatures. Sixty-eight, one-day old, Cobb500 males were separated into 4 treatments with 17 broilers each and fed in three phases: starter (d 1-10), grower (d 11-21) and finisher (d 22-35). A thermoneutral control (TC) group was reared with temperatures decreasing from 27.3 to 22.5 C from day 21 – 35. TC were fed a basal diet deficient in standardised ileal digestible (SID) M+C at 0.74, 0.65 and 0.61% in the starter, grower and finisher phases. The other 3 treatments were reared with ambient temperature of 27.4 C throughout day 21 to 35. A heat stress control group (HC) was fed the same basal diets as TC. Supplementation of basal diets with DL-methionine was utilised to create treatments that met (DLM1) or exceeded (DLM2) current SID M+C recommendations. The DLM1 diets contained 0.93, 0.81 and 0.76% SID M+C, while DLM2 diets contained 1.11, 0.81 and 0.76% SID M+C in the starter, grower and finisher phases. TC and HC treatments were compared with a T test to determine significant ($P > 0.05$) effects of heat stress. HC, DLM1 and DLM2 were analysed with a one-way ANOVA to determine significant ($P > 0.05$) effects of dietary M+C level in heat-stressed broilers. Tocopherols in the liver were significantly reduced from 29.0 nmol/g in TC to 23.3 nmol/g in HC. However, DLM2 treatment had significantly higher liver tocopherols than HC with 28.2 nmol/g. This may be due to the significantly higher liver glutathione in DLM2 (4.17 $\mu\text{mol/g}$) compared to HC (3.04 $\mu\text{mol/g}$) as glutathione can restore tocopherol radicals created during heat stress. COPs were higher in heat processed thigh meat from HC compared to TC, indicating that heat stress compromises broiler meat quality. The COPs 7 α -hydroxy-cholesterol and 7-keto-cholesterol in processed thigh meat of the DLM1

(268 and 398 $\mu\text{mol/g}$, respectively) and DLM2 (308 and 4555 $\mu\text{mol/g}$, respectively) treatments were significantly decreased when compared to HC (513 and 619 $\mu\text{mol/g}$, respectively). In conclusion, the current dietary SID M+C recommendations for broilers allow for sufficient glutathione production in high ambient temperatures, which not only ameliorates oxidative stress in the birds, but also prevents subsequent meat quality issues.

Key Words: sulfur amino acids, heat stress, broiler, methionine, meat quality

107 Effects of feeding varying levels of DL methionine on meat quality of broiler chickens. Anthony Pokoo-Aikins*¹, Jennifer R. Timmons², Byungrok R. Min², Samuel N. Mwangi⁴, Chongxiao Chen³; ¹*Toxicology & Mycotoxin Research Unit, USDA ARS, Watkinsville GA, Georgia, United States*, ²*Agriculture Food & Resource Sciences, University of Maryland Eastern Shore, Princess Anne, Maryland, United States*, ³*Poultry Science, North Carolina State University, Raleigh, North Carolina, United States*, ⁴*Agriculture & Applied Sciences, Alcorn State University, Lorman, Mississippi, United States*.

The amount of synthetic Methionine (MET) permitted by USDA in organic broiler feed is 2 pounds/ton of feed. Therefore, this study evaluated the effects of feeding dietary supplemental DL-(MET) on breast meat quality of broilers raised to a target weight of 2.72kg. Meat quality parameters investigated were color, pH, drip loss, water holding capacity, moisture uptake, cooking yield, texture, total antioxidant capacity (TAC), and lipid oxidation (LO). A total of 1,552 1-day-old Ross 708, sexed broiler chicks were randomly distributed to 32 pens, resulting in 8 treatments of 4 replicates with 44 males or 53 females/pen. A randomized complete block with a 2 x 4 factorial arrangement of treatments (sex x 4 MET levels 0, 0.5, 1.0, and 2.0g/kg feed) was used. The MET levels in the basal diets for starter, grower and finisher were, 0.37, 0.31 and 0.33%, respectively, while the MET+Cys levels in the same diets were, 0.69, 0.67 and 0.59%, respectively. Data were analyzed using GLMP for ANOVA. Mean differences were determined using Tukey's all-pairwise comparison test at $P \leq 0.05$. No significant interactions were observed between MET and sex on meat quality except for TAC and LO of raw breast meat stored for 10 days. Raw breast meat of male broilers fed 0g MET had higher ($P \leq 0.05$) LO levels at day 10 of storage than those of males fed 0.5g and 1g MET. Breast meat from male broilers had significantly higher redness, TAC, firmness, and toughness but lower yellowness ($P < 0.01$) than those from females. Broiler sex considerably affected the sensory quality of the meat. MET supplementation did not affect most of the meat quality parameters investigated in this study. However, birds fed 0.5g MET had significantly lower drip loss ($P < 0.01$) than those fed 1 and 2g MET. Males fed 0.5, 1 and 2g MET and females fed 0 and 0.5g MET had higher ($P \leq 0.05$) muscle

TAC than females fed 1 and 2g MET. Broilers fed the basal diet (0g MET) had significantly higher meat redness than broilers fed 1 and 2g MET. Regardless of sex, LO in cooked breast meat significantly decreased at days 0 and 3 of storage ($P < 0.01$) as MET supplementation levels increased to 1g MET and higher. However, LO levels in the cooked meat at day 7 of storage were not affected by the MET supplementation. Furthermore, breast meat from birds fed 1 and 2g MET had higher ($P < 0.01$) cooking yield (79.04 and 78.60%, respectively) than those fed 0 and 0.5g MET (66.18 and 68.03%, respectively). These results suggest that the supplementation of MET at 1g MET or higher for broilers can improve oxidative stability and muscle protein functionality and increase the cooking yield of their breast meat.

Key Words: synthetic methionine, raw meat, cooked meat, meat quality, broilers

108 Dietary sulphur amino acid level affects lipid peroxidation and subsequent meat quality in broilers fed diets high in polyunsaturated fatty acids. Rose Whelan^{*1}, Johanna Zeitz², Tamara Ehbrecht², Anne Fleischmann², Denise Gessner², Silvia Friedrichs³, Marion Sparenberg⁴, Klaus Failing⁴, Dieter Luetjohann³, Klaus Eder²; ¹*Animal Nutrition, Evonik Operations GmbH, Birmingham, United Kingdom*, ²*Institute of Animal Nutrition and Nutrition Physiology, Justus-Liebig-University Giessen, Giessen, Germany*, ³*Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, Bonn, Germany*, ⁴*Unit of Biomathematics and Data Processing, Faculty of Veterinary Medicine, Justus-Liebig-University Giessen, Gi, Germany*.

Diets rich in polyunsaturated fatty acids (PUFAs), were previously shown to induce lipid peroxidation in the tissue of broilers which may lead to cholesterol oxidation products (COPs) in broiler meat. COPs have been linked with cardiovascular disease and cancer in humans. Glutathione is the most abundant and important intracellular antioxidant and is synthesized from the essential sulphur amino acids, methionine and cysteine (M+C). This study investigated whether increased dietary M+C could inhibit oxidative stress and prevent meat quality issues in broilers fed diets high in PUFAs. Sixty-six, one-day old, Cobb500 males were separated into 3 treatments with 22 broilers each and fed in three phases: starter (d 1-10), grower (d 11- 21) and finisher (d 22-35). All three treatments were fed diets with linseed oil as a fat source rich in PUFAs. Dietary linseed oil inclusion was 2.76, 3.60 and 4.16% in the starter, grower and finisher phases, respectively. The first treatment was fed an M+C deficient basal diet (DEF) with SID M+C at 0.74, 0.65 and 0.61% in the starter, grower and finisher phases, respectively. The other 2 treatments were fed diets supplemented with DL-methionine to either meet (DLM1) or exceed SID M+C recommendations (DLM2). SIDM+C in the DLM1 diets were formulated at 0.93, 0.81, and 0.76% in the starter, grower and finisher phases. In the DLM2 treatment SID M+C was formulated at 1.11, 0.96 and 0.90%

for the starter, grower and finisher diets. Treatments were compared with a one-way ANOVA and significance determined at $P < 0.05$. Liver and muscle glutathione were significantly increased in both DLM1 (3.51 and 1.39 $\mu\text{mol/g}$) and DLM2 (3.28 and 1.55 $\mu\text{mol/g}$) compared to DEF (2.61 and 1.13 $\mu\text{mol/g}$). Thigh meat was heat processed at 170 C for 50 min and analysed for COPs by gas chromatography–mass spectrometry. COPS were lower in heat processed meat from DLM1 (10.5 nmol/g) and DLM2 (16.7 nmol/g) compared to DEF (30.3 nmol/g). Correlation statistics showed significant negative correlations ($r = -0.57$, $P = 0.007$) between levels of glutathione in breast muscle and COPs in heat processed thigh meat. This suggests that dietary M+C level as a substrate for the glutathione antioxidant system can prevent lipid peroxidation in the tissues and subsequent COP formation in meat. For most variables the current SID M+C recommendations met in the DLM1 diet were sufficient for glutathione production, inhibition of dietary PUFA induced oxidative stress and prevention from related meat quality issues.

Key Words: sulfur amino acids, PUFA, broiler, methionine, meat quality

109 Wnt/ β -catenin signaling pathway mediates methionine promotes feather follicle stem cells proliferation in yellow-feathered broiler chickens. Yi-jun Chen, Meng-jie Chen, Chun-qi Gao^{*}; *College of Animal Science, South China Agricultural University, Guangzhou, China*.

Yellow-feathered broiler chickens are facing different degrees of abnormal feather shedding in intensive poultry production systems, which reduces their commercial quality traits and consumer satisfaction. Feather integrity is related to dietary methionine (Met) levels. However, the mechanism of methionine controlling hair follicle development is still unclear. Therefore, this study aimed to explore the effects of Met on the cells proliferation, cells cycle, cells apoptosis, and its regulation on the Wnt/ β -catenin signaling pathway, via the isolation, culture and identification of feather follicle stem cells (FFSCs) of Yellow-feathered broiler chickens. The FFSCs was treated with commercial DMEM-medium (CON, the Met level is enough), and Met-free medium (no Met medium), respectively. MTT, cell counting and EdU assay were used to evaluate cells viability and proliferation, and then replenished Met (Met-rescue) or its metabolite S-adenosylmethionine (SAM) at different time points of cell proliferation. The cell samples were collected to analyze the cell cycle and cell apoptotic by flow cytometry, and detected the activity of Wnt/ β -catenin by Western blotting and immunofluorescence. Finally, iCRT3, a specific inhibitor of the Wnt/ β -catenin pathway, was used to verify the role of Wnt/ β -catenin in regulating the proliferation of FFSCs. The differences in the different groups were analyzed by one-way ANOVA followed by Duncan's multiple range test using SAS software. Results showed that Met-free for 6 h significantly decreased cells proliferation

and cells percentage of S phase fraction, increased the cells percentage in the G2 phase, and cells apoptotic, and inhibited the Wnt/ β -catenin signaling pathway ($P < 0.05$). After Met-rescue for 24 h, cells proliferation, cells cycle, cells apoptosis, and the Wnt/ β -catenin signaling were significantly improved and rescued to the levels of the CON group. However, SAM-rescue for 24 h failed to rescue the effects ($P > 0.05$). Furthermore, compared with the Met-rescue group, 20 mmol/L iCRT3 significantly decreased the cells proliferation, as well as the expression of downstream target proteins of β -catenin ($P < 0.05$). These results suggest that Met rather than its metabolite SAM accelerates the FFSCs cycle process, improves their proliferation and apoptosis through Wnt/ β -catenin signaling pathway in Yellow-feathered broiler chickens. [This work was supported by the National Natural Science Foundation of China (31972585)]

Key Words: broiler chickens, methionine, feather follicle stem cells, cell proliferation, Wnt/ β -catenin

110 DL-methionyl-DL-methionine transported by PepT1 improves intestinal epithelial integrity via intensifying frizzled7-mediated Wnt/ β -catenin signaling activity in domestic pigeon (*Columba livia*). Chen Zhong, Di-qing Tong, Chun-qi Gao*; *College of Animal Science, South China Agricultural University, Guangzhou, China.*

Currently, the mortality of young squabs is as high as 15%, which is critically involved in intestinal development retardation and low utilization of nutrients. Previous studies have shown that increased methionine (Met) provision above requirements promotes growth performance of poultry. Desirable dietary Met interventions targeting breeding pigeon seems to be a good strategy to improve intestinal health status in squabs. Therefore, this experiment was undertaken to investigate the effects of parental dietary DL-methionine (DL-Met) and DL-methionyl-DL-methionine (DL-Met-Met) supplementation on the intestinal development of young squabs and possible underlying mechanism. A total of 108 pairs of breeding pigeons and 432 1-day-old young squabs were randomly divided into 3 groups: the control group (CON) was fed a basal diet (CP=15%) and the experimental groups were fed a basal diet supplemented with 0.3% Met or Met-Met during all experiment. Each pair of breeding pigeons nourished 4 young squabs, and eight squabs from each treatment were randomly sampled on the 46th day of experiment. H&E staining, Western blotting and immunofluorescence were used to evaluate intestinal development. Autodock serials software and PyMOL was used for molecular docking. The t-test and Duncan's multiple range tests were used to determine the differences between two groups or more groups using SPSS. The results indicated that Met and Met-Met supplementation improved intestinal morphology and structure in squabs, as reflected by elevated relative intestinal weight of each small intestinal segment and villus height. Besides, Met and Met-Met supplementation exhibited significantly increased protein expression of

markers of cell proliferation (Ki67 and PCNA), villin and tight junction proteins (ZO-1 and Claudin-1) in jejunum. Moreover, the expression of Wnt/ β -catenin signaling pathway related proteins (Frizzled7, p-GSK-3 β , Active β -catenin, β -catenin, TCF4, c-Myc and Cyclin D1) and intestinal peptide transporter 1 (PepT1) in jejunum were considerably higher in the treatment group than that in the CON group ($P < 0.05$), with the Met-Met group having the highest expression. Consistently, the molecular docking results predicted the possibility that Met or Met-Met binds to membrane receptor Frizzled7. Collectively, parental dietary 0.3% Met and Met-Met supplementation improved the intestinal epithelial integrity in squabs by potentiating PepT1 expression and intensifying Frizzled7-mediated Wnt/ β -catenin signaling activity, and Met-Met is superior to Met. [This work was jointly supported by the Natural Science Foundation of Guangdong Province, China (2019B1515210001)].

Key Words: Domestic Pigeon, methionine, intestinal development, Wnt/ β -catenin signaling, PepT1

111 The optimum leucine requirement of female broilers in starter phase based on carcass traits. Saeid Amirdahri³, Hossein Janmohammadi³, Akbar Taghizadeh³, William Lambert^{*2}, Elham Asadi Someh¹, Majid Oliaee³; ¹*Department of Animal Science, University of Queensland, Queensland, Australia, Brisbane, Queensland, Australia,* ²*METEX NOOVISTAGO, Paris, France,* ³*Department of Animal Science, University of Tabriz, Tabriz, Iran (the Islamic Republic of).*

Leucine (Leu) is one of the so-called functional AA, which plays an important role on the activation of *de novo* protein synthesis. However, little is known about the optimum level of Leu in young female broiler chickens to support carcass traits. Therefore, the purpose of this study was to determine the optimal digestible Leu (d-Leu) in a ratio to digestible lysine (d-Lys) in female Cobb-500 broiler chicks based on carcass traits from 8 to 21 days of age, using broken-line models. A total of 540 female broiler chickens of the Cobb-500, were distributed in a completely randomized design with 6 treatments and 6 replicates of 15 birds each. To create Leu deficient diet, a basal diet (based on wheat grain-soy bean meal) was formulated to meet of all nutrients (Ca = 0.8, Ava-P = 0.4, d-SAA = 0.78, d-Thr = 0.76, d-Val = 0.9 % diet) according to Rostagno et al. (2011), with the exception of d-Leu and d-Lys levels. To avoid excessive Lys, 90% d-Lys (1.05%) was used. To obtain the different d-Leu to d-Lys ratio in treatment diets, crystalline glutamic acid in basal diet was gradually replaced by crystalline Leu, and six different ratios of d-Leu to d-Lys ratio of 0.93, 0.98, 1.03, 1.08, 1.13 and 1.18 were formulated in isonitrogenous (CP = 19 %) and isoenergetic (AME_n = 2,889 kcal/kg) diets. To evaluate the optimal d-Leu of female broiler chicks for maximum carcass traits, 12 chicks from each treatment were selected and slaughtered by Halal method on d 21 of age, and the weights of edible carcass, breast, thigh and abdominal fat were recorded. Body weight (BW) at the age

of 21 days varied from 709 g/bird in diet with d-Leu to d-Lys ratio of 0.93 to 805 g/bird in diet with d-Leu to d-Lys ratio of 1/13. Feed conversion ratio was not significantly affected by different ratio of d-Leu to d-Lys and varied between 1.51 and 1.48. In this study, edible carcass, breast and abdominal fat weights were quadratically respond ($P < 0.05$) to the increasing level of d-Leu. Using linear broken-line model, the optimal d-Leu to d-Lys ratio to maximize edible carcass, thigh and abdominal fat weights were estimated at 1.02, 1.00 and 1.02, respectively. The quadratic broken-line model was estimate the optimal d-Leu to d-Lys ratio for maximum edible carcass, breast, thigh and abdominal fat weights at 1.06, 0.99, 1.09 and 1.05, respectively. In the current study the optimal d-Leu to d-Lys ratio to maximize carcass traits of female broilers from 8 to 21 day of age, was estimated within 0.99 to 1.09; which were in line with the recommendations of previous reports on male broilers.

Key Words: broiler, amino acid, leucine, carcass trait, requirement

112 Isoleucine requirements for laying hens at peak laying phase via a dose-response study. Nadège Hervé¹, Josselin Le Cour Grandmaison², William Lambert², Simon Fontaine², Dinesh Kumar³, Alexandre Lebel⁴, Adeline Mathiaud⁵, Anthony Quant⁶; ¹Cooperative Research Farms, ²Ajinomoto Animal Nutrition Europe, ³Federated Cooperatives Limited, ⁴Sollio Agriculture, ⁵Mixscience, ⁶Kalmbach Feeds.

In recent years, livestock production has been identified as one of the major causes of environmental changes, due among others to nitrogen (N) excretions of animals. Reducing crude protein (CP) content in the diets of laying hens may be a lever for reducing N excretion as well as improving gut health of layers related to egg production. Still, to achieve efficient diets, precise knowledge of amino acids (AA) requirements is essential. Isoleucine (Ile) has been identified as one of the next limiting AAs for laying hens fed with corn-soy diets. Therefore, a dose-response trial was set up to finetune the Ile requirements of layers when reaching the peak laying phase. Seven treatments were distributed to 252 Dekalb white layers separated in 84 cages (3 hens per cage) with 12 repetitions per treatment. The trial lasted from 19 to 28 weeks of age (WOA). The seven treatments were formulated with a digestible lysine (dLys) level of 0.75% with 7 levels of digestible isoleucine/digestible lysine ratios (dIle/dLys; %): 60, 66, 72, 78, 84, 90 or 96. All other essential AA were above the assumed requirements. Feed intake (FI), egg weight (EW), egg mass (EM), laying rate (LR) and feed conversion ratio (FCR) were measured. As analysed chemical total value of Lys was abnormally higher in treatment 1 than in the other treatments (total Lys = 0.85 vs 0.77), data from this treatment was removed from data analysis. Diets were fed for 10 weeks, however, only the last 8 weeks were used to measure treatment effect, the 2 first weeks served for adaptation. Due to the strong quadratic shape of the curve

with absence of plateau, data were analysed only using quadratic regression models. Initial body weight (BW0), WOA were used as covariable in the model and dIle/dLys ratio as the continuous predictor of the response. Responses were FI, EW, EM, LR and FCR. Isoleucine level had significant effect ($p < 0.05$) on all responses except for FI for which it only tended to be affected ($p < 0.10$), WOA had significant effect ($p < 0.01$) on all responses, and BW0 had significant effect ($p < 0.01$) on FI, EW and EM. Statistical requirements for dIle/dLys were evaluated to be 83.5 considering FI (R^2 adj. =9.8%), 79.9 considering LR (R^2 adj. =8.5%), 84.1 considering EW (R^2 adj. =55.9%), 81.3 considering EM (R^2 adj. =43.8%), and 80.4 considering FCR (R^2 adj. =20.55%). From this trial, depending on the response factor of interest, it may be concluded that the ideal dIle/dLys ratio for laying hens at laying peak is 80%.

Key Words: Layer, Egg, Performance, isoleucine

113 Is the broiler response to dietary valine modulated by the same branched-chain amino acids as in piglets? Maroua Zouaoui¹, William Lambert^{*2}, Aude Simongiovanni², Marie-Pierre Létourneau-Montminy¹; ¹Sciences Animales, Université LAVAL, Quebec, Quebec, Canada, ²METEX NOOVISTAGO, Paris, France.

Branched-chain amino acids (BCAA), that include valine (Val), leucine (Leu) and isoleucine (Ile), share the same unique catabolism pathway, leading to strong metabolic interactions. Excess of dietary Leu was first shown in piglets to stimulate the degradation of all BCAA including Val and Ile, with negative consequences on feed intake and growth. The aim of this study was thus to quantify and compare the response of broilers and piglets to dietary Val and the impact of the two others BCAA, Leu and Ile, on this response thanks to a meta-analysis approach. The databases were composed of 23 articles published between 1999 and 2018 including 44 experiments and 239 treatments for broilers, and 16 articles published between 2001 and 2018 including 23 experiments and 126 treatments for piglets. Multiple regression models were fitted with the MIXED procedure of Minitab software with experiment as random effect. The Y variables were average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). For broilers, the Y variables were expressed in percentage of the highest level intra-experiment to make the values comparable between experiments (different ages and genetic lines). The main X variable was the dietary standardised ileal digestible Val level (ValSID) and the other ones were dietary SID Leu and Ile (LeuSID and IleSID, respectively). The response of ADG and ADFI to ValSID was linear and quadratic for broilers and piglets (Broilers, ADG: $P < 0.001$, $R^2=0.65$, ADFI: $P < 0.001$, $R^2=0.50$; Piglets, ADG: $P < 0.001$, $R^2=0.93$, ADFI: $P < 0.001$, $R^2=0.97$). Results showed that increasing dietary LeuSID reduced ADG and ADFI in broilers and piglets ($P < 0.05$) but also that the response to ValSID was stronger with high LeuSID levels for these 2 species (Interaction ValSID×LeuSID: Broilers, ADG and ADFI: $P < 0.001$;

Piglets, ADG and ADFI: $P < 0.05$). The response of ADG and ADFI to ValSID was not modified by IleSID. The FCR responded linearly ($P < 0.001$) and quadratically ($P < 0.001$) to ValSID, while LeuSID and IleSID have no effect on this response for both species. In conclusion, the current study showed that ADG and ADFI but not FCR response to ValSID is modulated by dietary LeuSID level. These results indicate that Leu is the most important regulator of BCAA metabolism that works similarly in broilers and pigs. This quantification will facilitate the decision-making on amino acid formulation constraints and the implementation of low protein diets for these two species.

Key Words: broiler, valine, interaction, BCAA

114 Impact of coccidiosis on ileal digestibility of amino acids in broiler chickens: a meta-analysis. Emily Kim^{*1}, Marie-Pierre Létourneau-Montminy², William Lambert³, Elijah Kiarie⁴; ¹*Animal Biosciences, University of Guelph, Guelph, Ontario, Canada*, ²*Département des Sciences Animales, Université Laval, Québec city, Québec, Canada*, ³*Metex Noovistago, Paris, France*, ⁴*Animal Biosciences, University of Guelph, Guelph, Ontario, Canada*.

Caused by apicomplexan protozoa *Eimeria*, avian coccidiosis is responsible for major economic losses in the global poultry industry. Sub-clinical *Eimeria* infections impair digestive tract capacity and barrier function leading to poor growth and feed efficiency. The objective of this study was to use a meta-analysis approach to evaluate and quantify the effect of an *Eimeria* infection on the apparent ileal digestibility (AID) of amino acids (AA) in broiler chickens. A database composed of 6 articles with a total of 21 experiments was built to look at effect of challenge and type of challenge (a mix of *Eimeria* spp. vs *E. acervulina*), while a sub database of 3 articles with a total of 15 experiments was used to evaluate the effect of dose of *E. acervulina*-challenged birds. For the overall database, regression models were fitted with the mixed model procedure in Minitab 19 with fixed effects of challenge, species, and their interactions. For the sub database, the mixed model procedure was used to fit regression models and identify a linear or quadratic response to dose. Challenge decreased AID ($P < 0.05$) of both dispensable and indispensable AA except for Trp. Specifically, the largest depression was observed for Cys, Thr, Tyr, Ala, and Val with the magnitude of difference of 8.7, 5.4, 5.2, 5.1, and 4.9%, respectively for challenged vs. unchallenged birds. The type of challenge affected ($P < 0.05$) AID of AA with exception of Cys, Tyr, Ala, Ser, Leu, Asp, Gly, and Pro. *E. acervulina* challenge had larger negative effects on AID of Ile, Leu, and Val. Moreover, increasing *E. acervulina* dose linearly decreased ($P < 0.05$) AID of all indispensable and dispensable AA except for Trp and quadratically decreased AID of all AA except Cys, Met, Arg, and Trp. The largest linear decrease due to effect of *E. acervulina* dose was seen in AID of Cys, followed by Ala, Val, Thr, and Ile. Although, AID of Trp was not affected by *E. acervulina* challenge,

mixed *Eimeria* species challenge decreased ($P < 0.05$) AID of Trp. In conclusion, the effect of *Eimeria* on AID of Thr was supported by the increased secretion of mucin associated with an *Eimeria* infection while Cys played a role in oocyst sporulation. In addition, the effect of *Eimeria* on branched chain AA was likely due to their involvement in the proliferation and production of immune associated cells and proteins. Overall, the results confirmed that an *Eimeria* infection negatively impacted AA digestibility/utilization. The ranking of the most affected AA suggested ground for nutritional intervention during sub-clinical field *Eimeria* infections or vaccination programs.

Key Words: broilers, amino acids, meta-analysis, digestibility, *Eimeria* challenge

115 Effect of full or partial replacement of soybean meal and crude protein reduction in the grower phase on the performance of broiler chickens. Ahmed Amerah^{*1}, Henk Enting¹, Josselin Le Cour Grandmaison², Simon Fontaine², William Lambert²; ¹*Cargill, Velddriel, Netherlands*, ²*METEX NOOVISTA Go, Paris, France*.

Decreasing dietary crude protein in broiler chickens is a strategy known to have multiple benefits such as reducing N excretion, positively affecting intestinal health and reducing foot pad lesions. In addition, there is interest to increase the diversification of feed raw materials for broiler diets and to use more local alternative feed ingredients. This study evaluated the effect of full or partial replacement of SBM and crude protein (CP) reduction in the grower phase (14-36d of age) on the performance of broiler chickens. At 14 days of age, 324 Ross 308 male broilers were randomly allocated into 6 dietary treatments with 6 pens per treatment. Experimental diets were: A: control at 20.7% CP with 21%-SBM; B: 18.7% CP & 0%-SBM, C: 19.7% CP & 0%-SBM; D: 19.7% CP & 5%-SBM; E: 19.7% & 10%-SBM; F: 19.7% & 15%-SBM. All diets were isoenergetic and iso-digestible Lys and all the essential amino acids were at least at the level of the assumed requirement. In the low CP diets, inclusion of feed-grade L-Val, L-Arg & L-Ile was needed to balance the diets. In the zero or reduced SBM treatments, SBM was mostly replaced by sunflower (SFM), rapeseed (RSM) and some corn gluten (CGM) meal. Average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR) and final body weight (BWf) were evaluated and statistically analysed by 1-way ANOVA using BW at 14d and block as covariate. Birds were individually weighed at d36 and the coefficient of variation (CV) was calculated and statistically analysed. Between diets B, D, E and F, treatments only differed by SBM inclusion from 0 to 15% and therefore SBM inclusion effects were tested for linear and quadratic response. From day 14 to 36, there was no significant ($P > 0.05$) difference in ADFI ($\pm 149.3\text{g/d}$), ADG ($\pm 105.8\text{g/d}$), FCR (± 1.41), BWf ($\pm 2803\text{g}$) or CV (± 8.1) across treatments. No significant ($P > 0.05$) quadratic response was observed for any studied parameters. Reducing dietary SBM inclusion linearly ($P < 0.05$) reduced ADFI and tended to reduce FCR

($P=0.062$). The results indicate that reducing dietary CP (by -1%pt and -2%pt) and replacing SBM with SFM, RSM and CGM is possible in the grower broiler diets without negative impact on growth performance. These findings could support stakeholders to increase diversification of feed ingredients as well as improvement of animal nitrogen efficiency.

Key Words: Broiler, low crude protein, soybean meal, amino acids

116 Effects of reducing dietary crude protein under two different rearing conditions on performance, foot pad dermatitis, meat yields & environmental impacts of Ross 308 broiler chickens. Charlotte Raybaud², Pierre Moquet², Nicolas Brevault², William Lambert^{*1}, Stéphanie Lecuelle¹; ¹*METEX NOOVISTAGO, Paris, France*, ²*Mixscience, Bruz, France*.

Lowering dietary crude protein (CP) has multiple proven benefits on environmental impacts & welfare indicators of broiler chickens. This study investigated the effects of low CP diets under two rearing conditions on growth performance, foot pad dermatitis (FPD), meat yields & environmental impacts. A total of 1,305 male Ross 308 were randomly allocated to a 3 x 2 factorial design with 3 CP levels (Control : CTR, CTR -1 pt and CTR -2 pts) and 2 rearing conditions (Optimal: O and suboptimal: S) as experimental factors. Each treatment was replicated 5 times. Dietary CP levels were 21.8, 20.3, 18.3 & 17.3%, in the starter, grower, finisher 1 & 2 CTR diets, respectively. Low CP treatments were formulated by reducing CP level by 1 or 2 pts in all phases. Soybean meal was gradually replaced by wheat and feed-grade amino acids (AA). All indispensable AA were adequately supplied at the level of assumed requirement, digestible Lys and AMEn were kept constant across treatments. Changes in rearing conditions were achieved by using Paracox vaccine at 0 and 9 d of age, removing coccidiostats, replacing wood shaving by long straws bedding material and increasing density from 15.4 to 18.1 bird/m² for S kept birds. A two-way ANOVA was used to compare groups and P-values <0.05 were deemed significant. Birds kept in S conditions had significantly lower 0-42 d growth performance (ADG -4.6 g/d, ADFI -5.74 g/d, corrected FCR +6 pts) and higher FPD scores than their O kept counterparts. Dietary CP had no significant effect on overall growth performance, irrespective of the rearing condition. Reducing CP level by 2 pts improved significantly mean FPD scores (+0.41 pt). Birds fed CTR -2 pts diets tended to have lower growth performance on the 0-21 d period (e.g. ADG -3.6 g/d) and had significantly lower breast meat yields (-0.8 pts) irrespective of the rearing conditions. Reducing CP showed a positive impact on environmental footprint per ton of chicken meat produced in a dose-dependent manner (e.g. climate change -5.7%, energy use -3.6%, land use -5.5% for CTR -1 pt vs CTR). Summarizing, the present study confirms the importance of technical management and demonstrates that reducing CP improves welfare and environmental outcomes of broiler

production without compromising on growth performance. Future research may be directed towards the alleviation of the negative effect of -2 pts CP diets on starting performance and breast meat yield.

Key Words: broiler, low protein, challenge

117 Impact of reducing dietary crude protein level on male turkeys performance. Roshan Adhikari^{*1}, Jason T. Lee¹, Randy D. Mitchell², Arturo Garcia³; ¹*CJ Bio America, Downers Grove, Illinois, United States*, ²*Perdue Farms, Salisbury, Maryland, United States*, ³*Cargill Provimi USA, Lewisburg, Ohio, United States*.

Reducing dietary crude protein (CP) while maintaining essential amino acid ratios using free amino acids improves litter condition, pellet quality, intestinal health, performance, reduces nitrogen excretion and diet cost in broilers. However, very limited research has been conducted to understand the effect of reducing CP in heavy turkey toms. The objective of the study was to determine the effect of reducing dietary crude protein while maintaining minimum essential amino acid ratios to lysine with the addition of free amino acids (threonine, valine, isoleucine, arginine, and tryptophan) in heavy turkey toms. Four treatments (Control, less 3%, less 6%, and less 9% of total CP levels as compared to Control) were allocated in a completely randomized design to 32 pens that housed 85 Hybrid toms per pen. An eight-phase NAE vegetable dietary program was formulated meeting or exceeding the current breeder recommendations except for CP levels and fed to toms for 133 days. Dietary protein reductions in the most restrictive treatment (less 9%) were reduced by 2.5% in the starter phase and reduced to 1.5% reduction in the WD phase. Bodyweight and FI were determined on days 42, 84, and 133. Data were subjected to one-way ANOVA using the GLM procedure in SAS. Tukey's test was used to compare the mean separation and considered significance at $P \leq 0.05$. On day 84, bodyweight of toms fed the lowest CP (less 9%) diet was higher than the toms fed the control ($P=0.049$) which corresponded with an increase in body weight gain from day 42 to 84 ($P=0.040$). At the end of the experiment (d133), no significant difference was seen in body weight ($P=0.292$) and FCR ($P=0.962$). The data indicate that toms fed reduced CP diets with a balanced amino acids ratio could experience the same benefits of crude protein reduction as broilers have with increased body weight gain during the early stages of growth. The results of this experiment demonstrate that reducing dietary CP in heavy turkey toms does not have any negative impacts on growth performance and FCR when dietary essential amino acids ratio are maintained.

Key Words: Turkey toms, reduced CP, non-bound amino acids, performance, amino acid ratios

118 Determining the digestible lysine requirement of Cobb MV x Cobb 500 FF broilers from 28-41 days of age and the effect of feeding varying digestible lysine levels on performance and processing yield. Dalton

Dennehy*^{GS 1}, Andrew Brown², Cesar Coto³, Leonel Mejia³, Kelley G. Wamsley⁴; ¹*Poultry Science, Mississippi State University, Mississippi State, Mississippi, United States*, ²*Poultry Science, Mississippi State University, Gordo, Alabama, United States*, ³*Cobb-Vantress, Siloam Springs, Arkansas, United States*, ⁴*Mississippi State University, Mississippi State, Mississippi, United States*.

Previous research using the Cobb MV × Cobb 500 FF broiler has determined the digestible lysine (dLys) requirement (Req), as well as the impact of varying dLys during the starter and grower phases on overall performance; however, the finisher phase has yet to be evaluated. Therefore, the objective of this study was to determine the dLys Req of Cobb MV × Cobb 500 broilers from d 28-41, as well as the effect of feeding varying finisher dLys levels on performance and processing. A 2 Sex (male or female) × 8 dLys (0.72, 0.80, 0.88, 0.94, 1.02, 1.10, and 1.18% dLys) factorial arrangement was utilized. Chicks were obtained from a commercial hatchery, vent sexed, and fed common starter, grower 1, and grower 2 diets (1.22, 1.12, and 1.02% dLys, respectively) consisting of corn and soybean meal. On d 28, birds were individually weighed and placed into one of 11 weight classes. One bird from each weight class was allocated to one of 96 floor pens containing used litter. Initially, three basal finisher diets were formulated: LOW (0.72% dLys); HIGH (1.18% dLys); and practical control (0.94% dLys). Intermediate dLys levels were obtained by blending calculated proportions of the LOW and HIGH basal diets. Birds and feeders were individually weighed at d 28, 35, and 40 to measure BWG, FCR, and ADFI. Additionally, on d 41, 3 birds/pen ± 100 g of the mean BW/pen were processed. In general, male broilers improved performance and processing yield when compared to female broilers (P<0.05). Sex × dLys was significant for d 28-40 BWG and FCR, whereas males fed >0.80% dLys improved BWG (P=0.049) and males fed 1.18% had decreased FCR but similar to males fed 1.10% dLys (P=0.012). For d 28-40 ADFI, a stepwise decrease was observed as % dLys increased (P<0.0001). Breast yield was greatest in birds fed 1.10% but similar to those fed 0.94, 1.02, and 1.18% dLys (P=0.015). Various regression models were tested to estimate dLys Req for each sex (quadratic regression, 95% of the asymptote; quadratic broken line). The dLys Req was estimated for fewer female metrics; however, for d 28-35 and 35-40 FCR, the estimated dLys Req ranged from 1.18 to 1.29% when using both models (P<0.05, R²=0.99). For female carcass weight, both models estimated the dLys Req to be 1.06% (P<0.05, R²=0.83). For male d 28-35 and 28-40 BWG, the estimated dLys Req ranged from 0.98 to 1.08% for both models (P<0.05, R²=0.91-0.95). For d 28-35 and 28-40 FCR, the dLys Req ranged from 1.17 to 1.32% for FCR using both models (P<0.05, R²=0.98-0.99). For male breast yield, the estimated dLys Req using both models ranged from 1.01 and 1.10% (P≤0.05; R²=0.77-0.80). Our study concludes that dLys Req from 0.98 to 1.32% optimized broiler performance and processing.

Key Words: amino acid, broiler, requirement, performance, digestible lysine

119 Determining the optimal digestible isoleucine to lysine ratio of Ross 708 x Ross YP male broilers from 28 to 42 days of age. Andrew Brown*^{GS 1}, Jason T. Lee², Roshan Adhikari², Keith Haydon², Kelley G. Wamsley¹; ¹*Poultry Science, Mississippi State University, Mississippi State, Mississippi, United States*, ²*CJ America Inc., Downers Grove, Illinois, United States*.

Previous research within our laboratory determined the optimal dIle:dLys ratio for the starter and grower phases to be 70 and 68% for Ross 708 x Ross YP male broilers. Thus, the objective of this study was to reevaluate the finisher phase in order to determine the optimal dIle:dLys ratio to maximize growth performance. On d of hatch, 2,400 chicks were equally allocated to 96 pens and a common starter (d0-14;70% dIle:dLys) and grower diet (d14-28;68% dIle:dLys) was provided. All birds were weighed at d28 and pen weights were equalized by block (12 replications/treatment). Experimental finisher diets (d28-42) were created from a common deficient (54% dIle:dLys) corn/soybean meal-based diet. After batching, half of the deficient was retained for the creation of the summit (84% dIle:dLys), through the addition of crystalline Ile. The remaining 5 experimental diets ranged from 59-79% dIle:dLys and were blended proportions of the deficient and summit diets. A practical control diet (PRAC-CON; 69% dIle:dLys) was manufactured separately to verify the blended control diet (50 deficient:50 summit blend; BLEND-CON; 69% dIle:dLys). For statistical analysis, the authors utilized the analyzed values and multiplied them by the calculated digestible estimate for Ile and Lys relative to the analyzed total estimate for each ratio. The adjusted analyzed ratios are: 58, 62, 65, 68 (BLEND-CON), 73, 74, 79, and 71 (PRAC-CON) % dIle:dLys. Birds were individually weighed, and feed intake was recorded on d42 to determine average BW, BW gain, bird uniformity, feed intake/bird (FI), and FCR. All dIle:dLys ratios were estimated using quadratic regression (QR; 95% of the asymptote), as well as linear and quadratic broken line models (LBL;QBL). The QR model for BW (P=0.004; R²=0.88) and BWG (P=0.010; R²=0.90) suggests the ratio for dIle:dLys to be 66%. When utilizing LBL and QBL, the ratio was estimated to be 62% (P=0.077, 0.098; R²=0.50, 0.45). Additionally, for d28-42 FCR, QR estimated the ratio to be 66% dIle:dLys (P=0.001; R²=0.97), LBL estimated it to be 63% (P=0.066; R²=0.74), and QBL estimated it to be 66% (P=0.066; R²=0.74). Based on the estimated ratios produced by the multiple regression models for all variables (BW, BWG, and FCR), these data suggest that the optimum grower dIle:dLys ratio for male Ross 708 × Ross YP broilers is 66%. Future research should utilize established optimums from previous phases to reevaluate the optimal dIle:dLys ratio of male Ross 708 × Ross YP broilers for the WD phase.

Key Words: Isoleucine, Optimal Ratio, Finisher Phase,

120 Determination of the optimal digestible arginine to lysine ratio in Ross 708 male broilers. Jose I. Vargas*^{GS 1}, Alejandro Corzo², Jason T. Lee³, Marcelo Silva², Wilmer J. Pacheco¹; ¹*Poultry Science, Auburn University, Auburn, Alabama, United States*, ²*Aviagen, Huntsville, Alabama, United States*, ³*C.J. Bio America, Downers Grove, Illinois, United States*.

This study was conducted to establish the optimal digestible arginine (dArg) to digestible lysine (dLys) ratio of YPM × Ross 708 male broilers from 1 to 14 d of age, 1 to 25 d of age (experiment 1), and 25 to 42 d of age (experiment 2) based on live performance, feed utilization, and processing yields. A total of 1080 (experiment 1) and 1008 (experiment 2) 1 d old male chicks, were sorted in 72 floor pens in a randomized block design, and subjected to diets with 6 dArg/dLys levels, with 12 replicate pens, each containing 15 and 14 chicks, for experiment 1 and experiment 2, respectively. The experimental diets were comprised of corn, wheat middlings, corn gluten meal, poultry meal, and soybean meal, and formulated to meet limiting amino acids requirements, except for dArg. Diets were formulated to 95% of the recommended dLys requirements to prevent overconsumption of dLys, and to ensure dArg would only be biologically efficient up to the slightly suboptimal lysine level. The calculated dArg/dLys value was formulated during all feeding phases studies to have a minimum level of 77, and 10-point increments were added to the basal diet in the form of L-arginine at the expense of an inert filler. Optimum dArg/dLys values for live performance and carcass traits for each experimental period, were calculated via quadratic broken line methodology based on adequacy of the fit, following the NLIN procedure of SAS 9.4. In experiment 1, the dArg/dLys ratio for optimizing BW gain and feed conversion ratio (FCR) was determined to be 106 for both variables from 1 to 14 d of age, and 105 and 108 from 1 to 25 d of age, respectively. In experiment 2, the optimum dArg/dLys value was estimated to be 129 and 116 for BW gain and FCR, respectively, during the 25 to 42 d period. Optimum dArg/dLys values for breast weight and breast yield were estimated to be 112 and 109, respectively. Absolute and relative weights of thighs increased linearly ($P < 0.001$) with progressive increases in dArg/dLys. The results of this study suggest that the optimum dArg/dLys value necessary to maximize growth performance and carcass traits increases as the bird matures, likely due to considerably higher maintenance values of dArg in broilers.

Key Words: arginine, lysine, growth performance, carcass traits, optimization

121 Efficiency of lysine and arginine utilization in broilers. Bernardo R. Nogueira*^{GS 2}, Luis Filipe V. de Freitas², Matheus D. Reis², Gabriel S. Viana¹, Nilva Sakomura²; ¹*Luke, Jokioinen, Finland*, ²*Animal Science, Paulista State University, Jaboticabal, SP, Brazil*.

To determine the efficiencies of Lysine (Lys) and Arginine (Arg) utilization, twelve dose-response assays were

conducted to describe male and female broiler chicken responses to Lys or Arg intake, as well as estimate the efficiency of both amino acids utilization for growth. In each assay, 1280 Cobb 500® broiler chicks (640 males and 640 females) in the starter (1 to 14 days old), grower (15 to 28 days old), and finisher (29 to 42 days old) phases were randomly assigned to one of 8 dietary treatments with four replicates of 20 birds each. The experimental diets were formulated using the dilution technique, where the concentration of digestible Lys investigated ranged from 8.70 to 17.5 g Lys/kg feed in the starter phase, from 7.8 to 15.7 g Lys/kg feed in grower phase, and 6.4 to 12.8g Lys/kg feed in finisher phase, and the content of digestible Arg ranged from 9.3 to 18.7 g Arg/kg feed in the starter phase, from 8.4 to 16.8 g Arg/kg feed in grower phase, and 6.8 to 13.7g Arg/kg feed in finisher phase. An additional treatment was included in all the assays to confirm whether Lys and Arg were the limiting amino acids in the feeds. The deposition of protein, Lys, and Arg in the feather-free body and feathers was measured using the comparative slaughter technique. The efficiency of Lys and Arg utilization was estimated, for each phase and gender, as the slope of the first-degree linear regression equation: $AA_{dep} = \beta + k \cdot AA_{Ti}$, where AA_{dep} is the investigated amino acid deposition, β is the intercept, and AA_{Ti} is the amino acid intake above maintenance requirement, calculated as the total intake of each evaluated amino acid minus its need for maintenance (36 mg of Arg and 45 mg of Lys). The efficiencies were compared using a linear regression with groups (GenStat, VSN International, 2017), the differences were considered statistically significant at 5% of probability. The efficiency of Lys utilization was estimated in 0.80, 0.81, and 0.81 for starter, grower, and finisher male broilers respectively, and in 0.78, 0.76, and 0.78 for females in the same phases. The efficiency of Arg utilization for male broiler chickens was estimated in 0.63, 0.63, and 0.62 in starter, grower, and finisher phases, respectively, while females had an efficiency of utilization for Arg estimated in 0.61, 0.63, 0.61 in starter, grower, and finisher phases, respectively. Our findings demonstrated that the efficiency of Lys and Arg utilization was not affected by either age or gender ($P > 0.05$). Overall, Lys and Arg were utilized for growth at 0.79 and 0.62, respectively.

Key Words: amino acids, comparative slaughter, efficiency of utilization

122 Influence of dietary valine and leucine levels on the live performance, carcass traits, and meat quality of Cobb MV × 500 males broilers from 32 to 45 d. Craig W. Maynard*^{GS 1}, Garrett Mullenix¹, Clay J. Maynard¹, Jason T. Lee², Shivi Rao³, Joseph Hiltz¹, Sara Orłowski⁴, Michael T. Kidd¹; ¹*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*, ²*CJ America-Bio, Downers Grove, Illinois, United States*, ³*Cobb-Vantress, Inc., Siloam Springs, Arkansas, United States*, ⁴*Poultry Science, University of Arkansas, Springdale, Arkansas, United States*.

Recent data has indicated that interactions among the branched-chain amino acids, specifically valine and leucine, can negatively influence broiler performance and carcass traits. Therefore, an experiment was conducted to observe the interactive effects between dietary valine and leucine on live performance, carcass traits, and Pectoralis major myopathies of Cobb MV × 500 broilers. A corn/soybean meal basal diet was formulated to contain Val/Lys and Leu/Lys ratios of 72 and 130, respectively, with a digestible lysine level of 1.05%. Graded amounts of feed-grade L-valine and L-leucine were added in order to produce 12 experimental diets consisting of a 4 (Val/Lys: 72, 77, 82, 87) × 3 (Leu/Lys: 130, 150, 170) level factorial design. Diets were fed to eight replicate pens of 21 male broiler chicks. Body weight gain, feed intake, and feed conversion were determined for the 32 to 45 d finisher period. At d 46, four birds per pen were processed for determination of carcass traits and the incidence and severity of woody breast in P. major fillets. All data were analyzed by two-way ANOVA using SAS 9.4. Statistical significance was considered at $P \leq 0.05$ and means were separated using a repeated t-test, when appropriate. No differences were observed for any live performance or carcass trait variable in response to dietary valine level, leucine level, or their subsequent interaction. Concerning woody breast, a significant interaction ($P < 0.05$) was observed for average score and the distribution of scores 1 and 2. These data indicate that within the valine and leucine levels tested, branched-chain amino acid antagonism did not negatively influence broiler performance and carcass traits. The appearance of an interaction between valine and leucine for woody breast parameters was surprising and the mechanism behind the interaction is currently unknown.

Key Words: Valine, Leucine, Branched-chain amino acids, Woody breast, Factorial

123 Effects of dietary sulfur amino acid supplementation on broiler chickens exposed to acute and chronic heat stress. Albaraa Sarsour*^{GS}, Mike Persia; *Virginia Tech, Blacksburg, Virginia, United States.*

Non-fatal heat stress (HS) can result in oxidative damage from increased reactive oxygen species. One method for alleviating these chronic effects of HS is the supplementation of sulfur amino acids (SAA) which can be metabolized to produce glutathione, one of the more important antioxidant systems used by poultry. Therefore, the objective of this experiment was to alter dietary SAA content of the diet of broiler chickens exposed to HS from 28 to 35 d to determine the effects on broiler performance, body temperature, intestinal permeability, and oxidative status. Serum concentrations of FITC-Dextran was used to quantify intestinal permeability and oxidative status was determined by reduced glutathione to oxidized glutathione ratio (GSH: GSSG). Four experimental treatments generated from 2 x 2 factorial arrangement of treatments consisting of HS (6 h at 33.3 °C followed by 18 h at 27.8 °C from 28 to 35 d of age) and Thermoneutral (TN: 22.2 °C

continuously from 28 to 35 d) and 2 dietary concentrations of SAA (0.80 and 1.04% digestible SAA that correspond to 100 and 130% of breeder recommendations). Diets fed during HS were formulated to contain 3,200 kcal/kg ME, 19.7% CP, and 1.06% digestible Lys and 0.71% digestible Thr. A total of 648 Ross 708-day-old male chicks were placed in 36 pens with 18 chicks/pen and 9 replicates per treatment. Data were analyzed as a 2 x 2 factorial in JMP 14 ($P \leq 0.05$). Cloacal temperature was increased by 1.7, 1.4 and 1.2 °C with HS at 28, 31, and 35 d, respectively ($P \leq 0.05$). No interaction effects were observed on broiler live performance ($P \geq 0.05$). As expected, HS reduced BWG by 92g and increased FCR by 11 points from 28 to 35 d of age, respectively ($P \leq 0.05$). The additional supplementation of SAA had no effect on live performance ($P > 0.05$). At 28 d of age, there was an interaction where supplementation of SAA to birds exposed to HS was able to reduce the intestinal permeability similar to the TN group ($P > 0.05$). The interaction was lost at 31 d, but HS still increased intestinal permeability ($P \leq 0.05$). By 35 d, broilers were able to restore intestinal function and intestinal permeability was not altered by HS or diet ($P > 0.05$). Additionally, SAA was able to reduce oxidative damage by reducing the GSH:GSSG ratio by 0.41 and 4.91 at 28 ($P = 0.08$) and 35 d ($P = 0.05$). These data suggest that intestinal damage occurs acutely and is possibly maximized within three days, but oxidative damage is more chronic building over the entire 7 d HS period. Increased dietary Met might have some protective effect on these responses to HS although no direct effects on bird performance were reported.

Key Words: Sulfur amino acids, Broilers, Heat stress, Intestinal permeability, Oxidative stress

124 Practical assessment of methionine supplementation regimen for 2 commercial broiler strains on 41 d performance and processing. Perri A. Purvis*^{UG1}, Andrew Brown¹, Dalton Dennehy¹, Kyle Smith², Kelley G. Wamsley¹; ¹Mississippi State University, Mississippi State, Mississippi, United States, ²Evonik Corporation, Kennesaw, Georgia, United States.

The current study was conducted with a commercial poultry integrator to help identify potential improvements for their methionine (Met) regimen (MR) utilized for two broiler strains they commonly use (fast growing (FG) vs. high yielding (HY)). Thus, two common sources of synthetic Met supplements were tested: a dry form, DL-Met (DLM), and a liquid form, Met Hydroxy Analogue (MHA). A 2 strain (FG or HY) x 6 MR (1 - 100% MHA to meet HY Breeder Spec for digestible TSAA; 2 - DLM at 65% of MHA used in 1; 3 - MHA at 150% MHA used in 1; 4 - DLM at 65% of MHA used in 3; 5 - MHA at 50% of MHA used in 1; and 6 - DLM at 65% of MHA used in 5) factorial arrangement was employed. All diets were analyzed and within target for DLM and MHA. Day old males (n=2700) were randomly assigned to treatment/pens by location (0.07 m²/bird); 9 replicates/treatment. Average BW, BW gain (BWG), feed intake (FI), and FCR were evaluated at d 17,

28, and 40. On d 41, 4 birds/pen (+/- 100 g of average BW/pen) were processed for carcass and breast weights. Data were analyzed using SAS; means were separated using Fisher's LSD where $P \leq 0.05$. Strain impacted performance ($P < 0.05$); overall, FG and HY birds finished behind breeder specs by ~2 and 1 d, respectively. Due to the strain x MR interactions being of primary interest and none detected ($P > 0.05$), only the impact of MR will be discussed. Birds fed MR5 had the highest d 17 BW (0.59 kg; $P = 0.027$) but similar to MR 2, 4, and 6; those fed MR3 had the lowest BW (0.56 kg), though similar to MR 1 and 2. Day 0-17 BWG, FI, and FCR were increased when feeding MR 5 ($P < 0.05$); MR4 had intermediate BWG and FI, but also the lowest FCR. Day 28 BW and 0-28 BWG was maximized when feeding MR5 and 6 as compared to MR1-4 ($P < 0.05$). However, MR5 had the highest 0-28 d FCR (1.42), followed by MR6 (intermediate), the lowest FCR was found for MR3 (1.38; $P = 0.001$). Day 40 BW and 0-40 BWG was maximized by feeding MR6 (2.56 kg), however birds fed MR5 were similar for both BW and BWG, and MR2 was similar for BW ($P < 0.05$). For 0-40 d FCR, MR3 had the lowest (1.51), while MR1, 2, and 4 had the next lowest (~1.53; $P < 0.0001$). Birds fed MR6 were intermediate (1.56), and MR5 had the highest FCR (1.57). Carcass weight was maximized by MR6, though similar to MR2; MR1, 3, and 4 had the lowest carcass weight ($P = 0.001$). Total breast weight was maximized by MR4, though MR2 and 3 were similar ($P = 0.002$). Birds fed MR5 had the lowest total breast weight, but similar to MR1, MR6 was intermediate. Data suggests MR6 birds compensated for reduced Met via increased FI and FCR. These data provide nutritionists with insight for MR application to optimize performance and economics and reveal no MR x Strain concerns under these parameters.

Key Words: amino acid, broiler, performance, methionine

125 An investigation into the influence of age on the standardized amino acid digestibility of wheat and sorghum in broilers. Mukti Barua*^{GS 1}, M. Reza Abdollahi¹, F. Zaefarian¹, T.J. Wester¹, C.K. Girish², Peter V. Chrystal³, V. Ravindran¹; ¹*Monogastric Research Center, School of Agriculture and Environment, Massey University, New Zealand, Palmerston North, Manawatu, New Zealand,* ²*Nutrition and Care, Animal Nutrition, Evonik (SEA) Pte. Ltd., Jurong Island, West Region, Singapore,* ³*Baiada Poultry Pty Ltd, Baiada, Pendle Hill 2145, New South Wales, Australia.*

The present study was conducted to determine the standardized ileal digestibility coefficients (SIDC) of nitrogen (N) and amino acids (AA) in wheat and sorghum at six different ages (d 7, 14, 21, 28, 35, and 42) of broilers. Two assay diets were formulated to contain 938 g/kg of each grain as the sole source of AA in the diet. Titanium dioxide (5 g/kg) was added as an indigestible marker. Each assay diet was fed to six replicate cages housing 14 (d 7), 12 (d 14), 10 (d 21), 8 (d 28), 8 (d 35), and 6 (d 42) birds per cage for four days prior to digesta collection from the lower half

of the ileum. The apparent ileal digestibility coefficients (AIDC) were calculated and standardized by using age-appropriate basal ileal endogenous AA losses determined in a previous study by feeding a N-free diet and collecting digesta on d 7, 14, 21, 28, 35, and 42. Data were analyzed by using GLM procedure of SAS with cage means as the experimental unit. Orthogonal polynomial contrasts were performed to determine the linear and quadratic effects of broiler age. In the case of wheat, AIDC of N and all AA increased (linear or quadratic, $P < 0.05$ - 0.001) with advancing age. No age effect was noticed on the SIDC of N, average of indispensable (IAA) and dispensable AA (DAA), though the average of total AA tended (linear, $P = 0.09$) to be increased by age. In sorghum, the AIDC of N, average of IAA and DAA were unaffected ($P > 0.05$) by age. The AIDC of Met, Trp, Cys, Asp increased linearly ($P < 0.01$) as the birds grew older. However, the SIDC of N, average SIDC of IAA, DAA and total AA were higher at d 7, reduced at d 14 and then plateaued. Among the IAA, the SIDC of Arg, His, Thr and Val decreased (quadratic, $P < 0.05$ to 0.01) with age. A linear decrease ($P < 0.05$ to 0.001) was observed in the SIDC of Ile, Leu, Lys, and average of IAA with higher values on d 7. The SIDC of all individual DAA (except for Cys) and the average of DAA decreased with age (linear or quadratic, $P < 0.05$ to 0.001) with higher values on d 7. Overall, the SIDC of AA increased in wheat and decreased in sorghum with advancing age. The current findings suggest that broiler age influences the AIDC and SIDC of most AA in both wheat and sorghum, and that the age effect may need to be considered in practical feed formulations. The age effect on the AA digestibility in broilers is variable depending on the grain type and specific AA. **Acknowledgement:** We acknowledge the "AgriFutures Australian Chicken Meat Program" for funding the project.

Key Words: age, broilers, amino acids, digestibility

126 Determining amino acid digestibility of soybean meal from different Midwest soybean varieties and growth performance when fed to broilers. Kara M. Dunmire*^{GS}, Michaela B. Braun, Caitlin E. Evans, Charles R. Stark, Chad B. Paulk; *Grain Science and Industry, Kansas State University, Manhattan, Kansas, United States.*

Two studies were conducted to determine apparent ileal digestibility (AID) of amino acids (AA) and growth performance of broilers fed soybean meal (SBM) from different soybean varieties. Both studies used 1 of 4 soybean sources varying in quality defined by analyzed crude protein (CP) content. Two sources consisted of soybeans from similar regions processed conventionally or experimentally. Other sources included a low quality and high-quality soybean, experimentally solvent extracted at Texas A&M University. Therefore, dietary treatments consisted of a conventionally processed SBM (CSBM; 47% CP) or experimentally processed SBM 42SBM (42% CP), 49SBM (49% CP), or 52SBM (52% CP). In Exp. 1, 240 one-day old

Cobb500 male broilers were placed in battery cages with 6 broilers per cage and 10 replicates per treatment. A common corn-SBM crumble starter diet was fed from d 0 to 10 with experimental diets fed from d 10 to 18 with SBM as the only source of AA. Dietary treatments were dextrose and SBM-based and consisted of 1 of 4 SBM sources included in diets formulated to 20% CP. On d 18, broilers were euthanized by CO₂ inhalation and ileal samples were collected to determine AID of AA. In Exp. 2, 360 one-day old Cobb500 male broilers were placed in battery cages with 6 broilers per cage and 15 replicates per treatment. Dietary treatments consisted of the same SBM used in Exp. 1 and were formulated to 90% digestible Lys, balanced using digestible AA values determined in Exp. 1. All data were analyzed using the GLIMMIX procedure in SAS 9.4, with cage as the experimental unit, cage location as the blocking factor and adjusted using Tukey-Kramer multiple comparisons. In Exp. 1, increasing the CP content of experimentally processed SBM from 42SBM, 49SBM, to 52SBM increased ($P < 0.05$) AID of total AA (80.3, 84.9, 87.3%, respectively; CSBM 87.6%), Arg, His, Lys, and Thr with no evidence for differences between AID of CSBM and 52SBM. Broilers fed CSBM, 49SBM, and 52SBM had increased ($P < 0.001$) Ile, Met, Phe, and Trp compared to 42SBM. The AID of Leu and Val increased ($P < 0.001$) with increasing CP with CSBM intermediate to 49SBM and 52SBM. In Exp 2., from d 0 to 18, ADG and d 18 BW increased ($P < 0.001$) in broilers fed CSBM (37.7; 688 g) compared to 42SBM (31.9; 635.7 g), 49SBM (32.6; 640.0 g), and 52SBM (31.1; 635.0 g). Broilers fed the CSBM (45.6 g) had increased ($P < 0.001$) ADFI compared to 42SBM (40.8 g) and 52SBM (40.0 g) with 49SBM (42.2 g) intermediate. In conclusion, SBM from high CP soybeans (52SBM) had increased amino acid digestibility compared to SBM from 42SBM and 49SBM with no differences between 52SBM and CSBM. However, broilers fed CSBM had improved d18 BW, ADG, and ADFI.

Key Words: broiler performance, amino acid digestibility, soybean meal

127 Effects of different heat stress models on ileal nutrient digestibility and molecular markers of protein metabolism. Jean-Rémi Teyssier*^{GS 2}, Aurélie Preynat¹, Pierre Cozannet¹, Elizabeth S. Greene², Sami Dridi², Samuel J. Rochell²; ¹Adisseo France S.A.S., Commentry, France, ²Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States.

Heat stress (HS) is a major stressor for commercial poultry, and we previously reported that feed intake (FI), body weight gain (BWG), and breast meat yield of broilers were decreased under HS compared with those under thermoneutral conditions. Pair-feeding (PF) revealed that approximately 81% of the reduction in BWG under constant HS was caused by decreased FI, with the remaining 19% directly associated with the physiological changes induced by HS. This experiment aimed to characterize the effects of

3 HS models, constant, cyclic and acute, on nutrient digestibility and markers of protein metabolism. A total of 720 Cobb 500 male chicks were allocated into 12 environmentally controlled chambers. From d20 to d41, 4 chambers were set to 35°C (**coHS**), 4 chambers were set to 35°C for 12h and 24°C for the next 12h (**cyHS**), and the 4 remaining were set to 24°C. Within the 24°C chambers, one-half of the birds was PF to equalize FI of coHS birds (**TN_{PF}**) and the other half was fed *ad-libitum* (**TN**). At d41, 16 TN birds were exposed to 35°C for 2 hours (**aHS**). Breast tissue and ileal digesta from 8 birds per treatment were collected on d41. For the cyHS groups, breast samples were taken before (**cyHS₁**) and after (**cyHS₂**) 6h of HS (35°C). RT-qPCR was performed on breast samples to evaluate the mRNA expression of different markers of protein metabolism. Ileal digesta was used to determine the apparent ileal digestibility (AID) of dry matter, ether extract, nitrogen (N), amino acids (AA) and energy. Data were analyzed by 2-way ANOVA and means were separated using Tukey's HSD. Compared with TN birds, the AID of N of coHS birds was reduced ($P < 0.05$; -5.3%). Only coHS reduced ($P < 0.05$) digestibility of essential AA (His, Leu, Phe, and Met; 2.7 to 6.3 percentage units) compared with TN birds. Both MAPK and IGF1/PI3K/AKT signaling pathways regulating mTOR were impacted. IGF1 was lowered ($P < 0.001$) with cyHS₁ and coHS, and upregulated in TN_{PF} birds compared to coHS birds. PI3K α was upregulated ($P = 0.002$) under aHS, cyHS₂, coHS and TN_{PF}. AMPK α 1, a marker of energy deficit, differed ($P < 0.05$) among treatments and was lowest for TN birds. Downstream of mTOR, S6K1 and MyoG were downregulated ($P < 0.01$) under coHS, and TN_{PF} and cyHS₁ also decreased expression of S6K1. MuRF1, a marker of muscle atrophy, was decreased ($P < 0.01$) under TN_{PF}, cyHS₁, and cyHS₂, but increased by coHS. These results suggest that coHS has a greater impact on AA digestibility and protein metabolism than cyHS. However, reduced AA digestibility appeared to only partly contribute to impaired performance induced by HS beyond that explained by lower FI, whereas a more important role may be played by alterations in regulatory mechanisms of protein metabolism.

Key Words: Heat stress, Protein, Metabolism, Breast, Digestibility

128 Effect of amino acid supplementation to reduced crude protein diets on growth performance, organ weights and nutrient digestibility of broiler chickens exposed to sub-clinical enteric health challenges. Marwa A. Hussein*^{GS 1, 2}, Farina Khattak¹, Lonneke Vervelde², Spiridoula Athanasiadou¹, Jos Houdijk¹; ¹SRUC, Edinburgh, United Kingdom, ²The Roslin Institute and R(D)SVS, University of Edinburgh, Midlothian, United Kingdom.

The ideal protein (IP) concept represents the ratio of digestible essential amino acid (AA) to lysine where all essential AA are equally limiting. However, sub-clinical

enteric challenges may increase specific AA requirements for immune responses and AA malabsorption compensation. Here, we assessed effects of threonine (Thr), arginine (Arg) and glutamine (Gln) supplementation to low crude protein (CP) diets on bird resilience to sub-clinical enteric challenges, organ weights and digestible CP intake. Ross308 day-old male broilers (14 birds/pen) underwent two challenge treatments factorially combined with four feeding treatments (n=9 pens per challenge-diet combination). Birds were either unchallenged (placed on clean litter; gavaged with water) or challenged (placed on reused litter; gavaged with 2500 *Eimeria maxima* oocysts at d14). Diets were high-CP (D1, 22.3% CP; Thr 0.86%; Arg 1.37%; Gln: 4.36%), low-CP (D2, 20.3% CP; Thr 0.86%, Arg 1.37%; Gln 3.67%), D2 with glycine (Gly) (D3, 21.7% CP; Thr 0.86%; Arg 1.37%; Gln 3.72%; Gly 2.23%), and D3 supplemented with extra Thr, Arg and Gln at the expense of Gly (D4, 21.9% CP; Thr 1.08%; Arg 1.70%; Gln 4.82%; Gly 0.72%). Birds were fed starter mash (d0 to d10) and grower pellets (d10 to d28), with D1 and D2 formulated to IP concept. On day 28, empty gizzard weight and ileal CP digestibility were assessed to calculate digestible CP intake (d25-28). Treatment effects were assessed via 2×4 ANOVA using d10 body weight (BW) as a covariate for d10-28 data; Tukey-adjusted multiple comparisons located effects at P<0.05. Feeding and challenge treatments did not interact. By d10, challenge increased BW (205 vs 212 g; s.e.d. 3) and reduced feed conversion ratio (FCR; 1.37 vs 1.31; s.e.d. 0.03), both P=0.018, whilst D1 birds were heavier than D2, D3 and D4 birds (219, 207, 204 and 202 g, respectively; s.e.d. 4; P<0.01). By d28, subsequent *E. maxima* challenge reduced final BW (1480 vs 1242 g; s.e.d. 17), increased d10 to d28 FCR (1.30 vs 1.40; s.e.d. 0.02) and reduced gizzard weight (29.5 vs 26.3 g; s.e.d. 0.68), all P<0.001. D1 birds were heavier than D2, D3 and D4 birds (1430, 1363, 1333 and 1318 g, respectively; s.e.d. 24) and had greater digestible CP intake (26.8, 23.9, 24.8 and 23.1 g/d, respectively; s.e.d. 0.71), all P<0.001. In our study, AA supplementation to IP based reduced CP diets did not improve resilience to sub-clinical enteric challenges, potentially due to AA imbalances. The reduced CP ration may have underperformed due to limiting non-essential AA, subclinical acidosis due to dietary electrolyte imbalance, or reduced ability to apply IP concept successfully during the starter phase.

Key Words: Ideal protein, broiler, reused litter, coccidiosis, resilience

129 Broilers administered a live coccidiosis vaccine or fed a chemical anticoccidial responded similarly to increased dietary amino acids contributed by soybean meal or feed-grade sources. Trevor Lee*^{GS 1}, Jason T. Lee², Samuel J. Rochell¹; ¹University of Arkansas, Fayetteville, Arkansas, United States, ²CJ America -Bio, Inc., Downers Grove, Illinois, United States.

This experiment evaluated responses of broilers under different coccidiosis control programs when fed elevated

amino acid (AA) concentrations achieved with increased dietary soybean meal (SBM) or feed-grade essential AA. Ross 708 male chicks were allocated in a 2×4 factorial design (24 birds/pen, 12 replicate pens/treatment) with 2 coccidia control methods (1x live vaccine dose or in-feed zoalene) and 4 experimental diets fed during the starter (0-12 d) and grower (12-26 d) periods: 1) a control diet (CTL), 2) an increased AA density diet containing increased SBM (H-SBM), 3) an increased AA density diet containing CTL-level SBM but increased feed-grade Met, Lys, Thr, Val, Ile, and Arg (H-FG), or 4) the CTL with only Thr, Val, and Arg increased by feed-grade sources to the same levels as the H-SBM and H-FG diets (CTL). Digestible Lys was 1.24 and 1.11% for the CTL diet and increased in the H-SBM and H-FG diets by 6 and 7% in the starter and grower phases, respectively, with other digestible AA/Lys ratios at or above breeder specifications. Common diets were fed during the finisher 1 (26-33 d) and 2 (33-40 d) phases. Feed intake (FI), BW gain (BWG), and FCR were measured during each phase, and fecal oocysts were counted at d 12 and 26. Intestinal segment length and weight were assessed at d 12. At d 40, 6 birds/pen were randomly selected for processing. Data were analyzed by a 2-way ANOVA and means separated by Tukey's test (P≤0.05). No interactions (P>0.05) between coccidia control method and diet were observed. At d 12, oocyst counts and intestinal length and weight were increased (P<0.001) with vaccination, with no diet effect. From d 0-26, vaccination impaired (P<0.001) BWG and FCR (1.19 vs. 1.22) compared with zoalene, but FI (P>0.05) was unaffected. Broiler BWG and FCR were better (P<0.001) for the H-SBM and H-FG groups compared with the CTL and CTL+ groups. Broiler FI was similar (P<0.001) among the CTL, H-SBM, and H-FG groups but reduced with CTL+. From d 0-40, FCR, but not FI or BWG, was impaired (P<0.001; 1.55 vs. 1.56) by vaccination compared with zoalene. Broiler FI (P<0.001) was highest for H-SBM and H-FG, moderate for CTL, and lowest for CTL+. Birds fed the H-SBM and H-FG diet had improved (P<0.001) BWG and FCR compared to those fed CTL and CTL+. Coccidia control method did not affect (P>0.05) deboned part yields. Breast meat yield (P<0.001) was greater for the H-SBM and H-FG groups than for the CTL and CTL+ groups. In conclusion, performance and yield of broilers given a vaccine or in-feed zoalene benefited from increased dietary essential AA regardless of source (SBM or feed-grade AA). However, these benefits were not observed when only ratios of Thr, Val, and Arg were increased.

Key Words: vaccination, coccidiosis, amino acids, protein density, broiler

130 Impact of *Eimeria* challenge on ileal endogenous losses and standardized ileal digestibility of amino acids in single and mixture of feed ingredients fed to broiler chickens. Emily Kim*^{GS 1}, John Barta², William Lambert³, Elijah Kiarie⁴; ¹Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, ²Pathobiology, University of Guelph, Guelph, Ontario, Canada, ³Metex Noovistago,

Paris, France,⁴Animal Biosciences, University of Guelph, Guelph, Ontario, Canada.

The negative effect of an *Eimeria* challenge on amino acid (AA) digestibility has been investigated, however, such studies primarily reported apparent ileal digestibility (AID) AA in diets. Thus, an experiment was conducted to determine standardized ileal digestibility (SID) of AA in major poultry feed ingredients fed to broiler chickens challenged with *Eimeria*. A total of 840 male day-old Ross x Ross 708 chicks were fed a commercial starter diet for 8 days. On day 9, birds were weighed, placed in 82 cages (10 birds per cage), and allocated to either a nitrogen-free diet (NFD) or one of the 6 test diets formulated to contain a single or mixture of feed ingredients as the sole source of AA (n=12). The test diets were: 1) corn, 2) wheat, 3) soybean meal (SBM), 4) pork meal (PM), 5) corn, SBM, and PM (CSP), and 6) wheat, SBM, and PM (WSP). All diets had 0.3% TiO₂ as the indigestible marker. On day 10, 6 replicates per diet were challenged with 1 mL of *E. acervulina* and *E. maxima* mixture via oral gavage and the other 6 replicates were given sham. On day 15, birds were bled for plasma AA and necropsied for intestinal lesion scores, and ileal digesta samples. The data was analyzed using a 3-way ANOVA, with treatment, challenge, and diet completeness (single vs. mixture) as fixed factors. Challenge ($P < 0.05$) decreased plasma concentration of

Arg, His, Thr, Asp, Gln, and Tyr and increased concentration of Lys, Ile, Leu, and Val. There was a challenge and diet completeness interaction ($P < 0.05$) on duodenum and jejunum lesion scores with challenged birds fed the mixed diet exhibiting more severe lesions compared to birds fed the single ingredients. The challenge increased ($P < 0.05$) basal endogenous losses of all AA except for Ala. There was a challenge and diet completeness interaction ($P < 0.05$) on total ileal endogenous flow (IEF) of AA with exception of His, Met, Ala, Asp, Cys, Gly, Pro, and Ser, such that challenged birds fed the mixed diets, particularly WSP, had higher IEF of AA compared to the single ingredients. Consequently, challenge decreased ($P < 0.05$) SID of most AA except for Met, Asp, and Cys with the largest impact seen on Lys, His, Ser, and Thr. An effect ($P < 0.05$) of diet completeness was seen on SID of most AA with exception of Arg, Thr, Asp, and Cys, such that values were higher in the mixed compared to the single ingredient diets. In summary, the effect of an *Eimeria* challenge and diet completeness can significantly influence ileal AA digestibility. The results showed that while challenge had a greater impact on the mixed diets, this may be due to AA intake which may influence *Eimeria* pathogenicity.

Key Words: amino acid, endogenous loss, SID, *Eimeria*, broilers

Metabolism and Nutrition: Enzymes

131 Effect of a novel consensus bacterial 6-phytase variant on phytate degradation in broilers fed diets containing different phytate levels. Abiodun Bello*¹, Yueming Dersjant-Li¹, Trine Christensen², Mehdi Toghyani³, Peter V. Chrystal⁴, Sonia Liu^{3,5}, Peter H. Selle³; ¹Danisco Animal Nutrition - IFF, Oegstgeest, 2342 BH, Netherlands, ²Danisco Animal Nutrition Aps - IFF, Brabrand, DK-8220, Denmark, ³School of Life and Environmental Science, Faculty of Science, The University of Sydney, Sydney, New South Wales, Australia, ⁴Baiada Poultry, Pendle Hill, New South Wales, Australia, ⁵Poultry Research Foundation, The University of Sydney, Camden, New South Wales, Australia.

This study evaluated the effect of a novel consensus bacterial 6-phytase variant (PhyG) on apparent ileal digestibility (AID) of phosphorus (P) and degradation rate of phytic acid (inositol hexa-phosphate, IP₆) in broilers. A 3 × 5 factorial arrangement which was based on 3 phytate-P (PP) levels (low: 2.45 g/kg, mid :2.95 g/kg or high:3.45 g/kg) and 5 PhyG doses (0, 500, 1000, 2000 and 4000 FTU/kg) was utilized. A total of 1,800 (Ross 308) day-old male chicks were randomly allocated to 90 battery cages with 6 replicate cages per treatment. Diets were based on wheat, corn, soybean meal, rapeseed meal and rice bran with Cellite (20 g/kg) as indigestible marker and were fed ad lib in pellet form in two phases (starter 0-10d: 2.60 g/kg dig P and 7.6 g/kg Ca and grower 11-21d: 2.10 g/kg dig P and 6.4 g/kg Ca). On day 21, ileum samples were collected from six birds per cage, pooled per cage for measuring P digestibility and IP₆ degradation. Data were analyzed as 3x5 factorial arrangement and phytase dose response was tested using exponential curve fitting (JMP 14). The analyzed PP content was 2.98, 3.45 and 3.85g/kg respectively in low, medium and high PP diets. No interaction between phytase dose and PP level was seen for ileal IP₆ content and IP₆ degradation. Increasing phytase dose exponentially (P < 0.0001) reduced ileal IP₆ content and increased IP₆ degradation regardless of PP levels. At 4,000 FTU/kg PhyG, the ileal IP₆ content was reduced (P<0.05) from 2.45% to below limit of quantification at the low PP, and from 2.52% to 0.14% at mid PP and 2.81% to 0.16% (freeze dried-DM basis) at mid and high PP level, respectively, which corresponded to IP₆ disappearance of 100%, 97.2% and 97.1% at each of low, mid, and high PP, respectively. At the 2,000 FTU/kg, the IP₆ disappearance was 97.2, 92.7, 92.2% respectively at low, medium and high phytate P, which culminated into 2.82, 3.05, 3.36 g/kg of phytate P release estimation based on the sum of IP₃₋₆ degradation for low, mid and high phytate diets, respectively. Phytase dose and PP level interaction was found for both AID P and ileal digestible P as g/kg. Phytase increased (P < 0.001) ileal digestible P (g/kg) and the improvement in response to each phytase dose was higher in the high PP diets than in the medium or low PP diets. In conclusion, while the supplementation of PhyG at 2,000 FTU/kg dose degraded

over 92% of phytate at each PP levels, the use of the phytase in diet up to 4,000 FTU/kg exponentially increased ileal IP₆ degradation in commercial broiler diets. The usage of the novel consensus bacterial 6-phytase variant in commercial broiler diets enables optimal phytate P degradation and utilization in broilers.

Key Words: broiler, phosphorus, novel consensus bacterial 6-phytase variant, inositol hexa-phosphate degradation, digestibility

132 A novel consensus bacterial 6-phytase variant improved growth performance and feed efficiency and lowered production cost in turkeys. Abiodun Bello*¹, Yueming Dersjant-Li¹, Arun Kumar²; ¹Danisco Animal Nutrition - IFF, Wilmington, Delaware, United States, ²School of Agriculture and Food Science, University of Queensland, Gatton, Queensland, Australia.

The effects of a novel consensus bacterial 6-phytase variant (PhyG) were evaluated in turkeys fed diets with nutrient reduction. Three hundred Nicholas male turkey poults (10 birds × 10 pen replicates) were assigned to three treatments. Diets were based on corn, soybean meal, canola meal, and wheat bran and were fed in four dietary phases of 0-21, 22-42, 43-63 and 64-84 d. The diets were a nutrient adequate positive control (PC); a negative control reduced by 0.18% available P, 0.21% Ca, 74.5 kcal/kg ME, 0.02-0.06% dig AA, and 0.05% Na (based on 2,000 FTU/kg phytase contribution: NC) vs PC; and the NC supplemented with PhyG at 2,000 (NC+PhyG) FTU/kg. Growth performance, feed efficiency, and production cost were determined on the cumulative 0-42d and 0-84d. The study was conducted in a completely randomized setting, while the generated data were analyzed using the Fit Y by X function of JMP 15.1 and treatment means were compared using Tukey HSD test. The 0-42d body weight gain (BWG) was reduced (P<0.001) by the NC (2.88 kg) vs the PC (3.29 kg), which was completely restored by the NC+PhyG (3.26 kg). The 0-42d FCR was worsened (P<0.05) by the NC (1.598) vs the PC (1.484), while the FCR was improved by NC+PhyG (1.424) vs each of the PC and NC diets (P<0.05). The 0-42d feed cost per kg BWG was decreased (P<0.001) by NC+PhyG (0.370 USD/kg) compared to the PC (0.408 USD/kg). Relative to the 0-84d BWG by the PC (11.11 kg), BWG was also decreased (P<0.001) by the NC (9.97 kg) and restored by the NC+PhyG (10.91 kg). The 0-84d FCR was also worsened (P<0.05) by the NC (1.990) vs the PC (1.930), and it was improved (P<0.05) by NC+PhyG (1.912). Feed cost per kg BWG for the 0-84d was also lowered by NC+PhyG (0.487 USD/kg) relative to the PC (0.520 USD/kg). These findings demonstrated the efficacy of the novel consensus bacterial 6-phytase variant to support efficient production of turkeys. The PhyG supplementation at 2,000 FTU/kg in commercial turkey diet compensated for reduction of 0.18% in available P, 0.21% in Ca, 74.54 kcal/kg in ME, 0.02-0.06% in dig AA, and 0.05% in Na, maintained or improved

growth performance and feed efficiency compared to positive control, resulted in production cost saving through 84 d of age.

Key Words: Turkey, phosphorus, growth performance, feed efficiency, economic benefit

133 Performance and tibia ash response of Ross 708 broilers to increasing concentrations of two commercial phytase products post pelleting. Kristina M. Bowen*¹, Elizabeth Lynch¹, Tim Boltz¹, Victoria Ayres¹, Mark Jackson², Joe Moritz¹; ¹Nutritional and Food Sciences, West Virginia University, Morgantown, West Virginia, United States, ²Huvepharma, Peachtree City, Georgia, United States.

Mixer added phytases must retain efficacy post conditioning and pelleting. Heat from saturated steam and friction upon pellet die extrusion may deactivate phytases. In addition, increasing phytase concentrations in diets may increase nutrient release from phytate. The objective of this study was to assess two commercially available phytases that were concentrated at 500, 1,000, or 2,000 FTU/kg in mixed mash post steam conditioning at 82°C for 30 sec and extrusion through a 4.8 x 38mm pellet die on 0 to 44d Ross 708 male broiler performance and tibia ash responses. The phytase products were Quantum Blue, derived from *E. coli* and expressed in *Trichoderma Reesei*, and Optiphos Plus derived from *E. coli* and expressed in *Pichia pastoris*. A 3 (Phytase level) × 2 (Phytase product) factorial arrangement of treatments within a randomized complete block design was utilized. A positive control and negative control diet were also manufactured and analyzed within a multiple comparison. The negative control diet and diets containing phytase contained available phosphorus and calcium levels 0.2% less than the positive control. Crumbled and pelleted diets were fed to 8 replicate pens of 30 chicks in three phases. Live performance, D20 and D44 tibia ash, and D44 hot boneless, skinless breast weight were measured. Analyzed phytase activity did not drop below 75% of the formulated values for any treatment within each feeding phase. Analyzed phytase activity did not differ among products. Day 44 live weight gain and both D21 and D44 tibia measures increased for all phytase concentrations relative to the negative control ($P < 0.05$). Phytase products did not differ in effect on live performance or breast yield with the exception of live weight at 20 days where birds provided with Optiphos were significantly heavier ($P < 0.05$). Day 20 tibia ash increased for 1,000 and 2,000 FTU/kg concentrations compared to 500 FTU/kg concentration ($P < 0.05$). There was a phytase product by phytase level interaction for D44 tibia ash ($P < 0.05$). Optiphos Plus increased D44 tibia ash to the highest treatment level with concentrations above 500 FTU/kg ($P < 0.05$), while Quantum Blue D44 tibia ash did not respond to varying concentration resulting in a higher overall tibia ash percentage for Optiphos Plus ($P < 0.05$). The assessed phytase products showed similar benefit to live performance and breast yield but varied in response to D44 tibia ash at 1,000 and 2,000

FTU/kg concentrations.

Key Words: Phytase, Mixer added, Tibia ash, Live bird performance, Thermostability

134 Effect of two commercially available phytases, supplemented at two different dose rates, on performance of broilers fed diets reduced in minerals, energy and amino acids. Gilson A. Gomes*¹, Xavière Rousseau¹, Zuzanna Wisniewska², Sebastian Kaczmarek²; ¹AB Vista, Marlborough, United Kingdom, ²Department of Animal Nutrition, Poznan University of Life Sciences, Poznan, Poland.

Even if the use of phytase in broiler diets is ubiquitous, there is still opportunity to optimize its usage through defining best dose rate via phytate-P analysis and taking advantage of the extra-P benefits of phytase supplementation thereby reducing broiler production cost. Eight hundred and twenty-eight male day-old Ross 308 were assigned to 7 treatments as follows: Positive control (PC), formulated to be nutritionally adequate in all nutrients; another positive control diet was also added to the trial, this time with the inclusion of 500 FTU/kg Phy QB (PC 500) and formulated taking into consideration its mineral matrix contribution; a negative control diet (NC) reduced by 0.22% avP, 0.24% Ca, 0.05% Na, 75kcal/kg AME, 0.025% dLys and other amino acids was fed and used as basal to which two commercially available phytases (Phy QB and Phy AP) were supplemented at either 750 or 1,500 FTU/kg. Diets were wheat, corn, soybean meal, rapeseed meal and sunflower meal based, fed as pellet (crumbled at starter phase), and feed and water provided ad libitum. Mortality was recorded daily, with livability being calculated (Liv), and weight of dead birds recorded. Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), mortality corrected feed conversion ratio (FCR) and body weight corrected FCR (bwcFCR) were calculated. Data were submitted to ANOVA (Livability analyzed using Chi-Square) and means separated using Student's T-test ($P < 0.05$). Additionally, linear and logarithmic orthogonal contrasts were tested using the NC, and the two different dose rates of each phytase tested, taking into consideration the analyzed values of phytase in the feed. Significance is accepted when $P \leq 0.05$, while trends are discussed when $P < 0.10$. Livability was not affected by treatments in any stage of the trial ($P > 0.10$). Birds fed the NC diet showed reduced performance (BW, ADG and ADFI) during the whole trial ($P < 0.05$). Both phytases improved performance of birds at all tested ages (BW, ADG, ADFI) in a linear and logarithmic manner, and for most of the variables the logarithmic equations described data better than the linear ($P < 0.05$). When looking at the logarithmic regressions, Phy QB showed a steeper slope when compared to Phy AP on BW, ADG and ADFI for all ages. The bwcFCR tended to be affected by dietary treatments ($P = 0.06$), and Phy QB was the only phytase capable to improve in a linear manner bwcFCR ($P < 0.05$). In conclusion, Phy QB was the only phytase to recover performance to similar levels of positive

control fed birds when supplemented at 1,500 FTU/kg in a negative control diet reduced by 0.22% avP, 75 kcal/kg AME, 0.025% dLys and other nutrients.

Key Words: linear contrast, logarithmical contrast, body weight corrected FCR, production cost, performance

135 Application of Butiauxella phytase and a combination of xylanase and β -glucanase improved long-term performance, egg quality, and production cost of laying hens. Emma White^{*1}, Abiodun Bello³, Leon Marchal³, Rachael Hardy¹, Ceinwen Evans¹, Julien Kanarek³, Arun Kumar²; ¹Danisco Animal Nutrition- IFF, Marlborough, SN8 1NY, United Kingdom, ²School of Agriculture and Food Science, University of Queensland, Gatton, Queensland, Australia, ³Danisco Animal Nutrition (NL) - IFF, Oegstgeest, 2342 BH, Netherlands.

The effects of a Butiauxella phytase (PHY) with or without a combination of xylanase and β -glucanase (XB), with application of substrate-based energy and nutrient matrices, were evaluated on performance, egg quality, and production cost in laying hens from 24-72 weeks of age (woa). At 24 woa, 576 ISA Brown hens (4hens \times 48 cage replicates) were assigned to three dietary treatment groups. Diets were based on corn, wheat, soybean meal, canola meal, and rice bran. Three commercial layer diets were reduced in 1) minerals only (MO) by 0.18% available P (AvP) and 0.20% Ca with 600 FTU/kg PHY inclusion (MO+PHY); 2) MO + energy and amino acids (FULL) 75 kcal/kg ME, and 0.01-0.04% dig AA with 600 FTU/kg PHY inclusion (FULL+PHY); and 3) FULL + 63 kcal/kg ME (COMBO) with 600 FTU/kg PHY and XB (616 U/kg xylanase and 76 U/kg β -glucanase) inclusion (COMBO+PHYXB). Whilst Phytate P was maintained in all diets (0.35%), soluble NSP was 1.83% in MO+PHY, 1.86% in FULL+PHY and 1.91% in COMBO+PHYXB. Egg production was recorded daily and feed intake, egg weight and egg quality were recorded every four weeks. Bodyweight was measured every eight weeks. Data was analysed across age and treatment by analysis of variance. Means were compared using Tukey's test. Average hen-day egg production (24-72 woa) was increased ($P < 0.001$) in the COMBO+PHYXB group (95.2%) compared to FULL+PHY (94.1%) and MO+PHY (93.4%). Average egg mass (24-27 woa) was increased ($P < 0.001$) in the COMBO+PHYXB (62.9 g) group, vs FULL+PHY (61.7 g) and MO+PHY (61.3 g). The COMBO+PHYXB hens maintained FCR at similar level vs FULL+PHY and MO+PHY hens. There was no effect of diet ($P > 0.05$) on liveability, bodyweight change, eggshell breaking strength, albumen height, yolk colour, abnormal, dirty, peewee, shell-less or soft-shelled eggs. COMBO+PHYXB tended to improve ($P = 0.054$) Haugh unit compared to MO+PHY. Egg shell thickness was increased ($P < 0.001$) in the COMBO+PHYXB hens (0.504 mm) compared to FULL+PHY (0.500 mm) and MO+PHY (0.500 mm). Similarly, feed cost/dozen eggs was further lowered (by 3.1% and 6.1%) in the COMBO+PHYXB group (€0.31) compared to FULL+PHY (€0.32) and MO+PHY groups

(€0.33). The supplementation of PHY at 600 FTU/kg and XB in laying hen diets, reformulated according to substrate-based matrices, efficiently improved productivity performance, egg quality and lowered production cost from 24 to 72 woa.

Key Words: Layers, substrate-based matrix application, xylanase, β -glucanase, phytase

136 Feeding broilers total inorganic phosphorus free diets with phytase maintained normal performance as shown in 4 independent trials. Leon Marchal^{*1,2}, Abiodun Bello², Eric B. Sobotik³, Gregory S. Archer³, Enric Esteve⁴, Carla R. Creus⁴, Cees Kwakernaak⁵, Ellen v. Eerden⁵, Yueming Derjant-Li²; ¹Animal Nutrition, Wageningen University & Research, Wageningen, Netherlands, ²IFF, Oostgeest, Netherlands, ³Poultry Science, Texas A&M University, College Station, Texas, United States, ⁴Animal Nutrition, IRTA, Constanti, Spain, ⁵Poultry Nutrition, Schothorst Feed Research, Lelystad, Netherlands.

There is a global growing sustainability awareness on the proper use of finite resources like inorganic phosphorus (P) and reduction of pollution. Broiler meat production uses millions of tons of inorganic P, with each ton emitting ~ 750 kg CO₂. In 4 independent studies it was investigated if it is possible to feed broilers a diet without any inorganic P and have normal growth characteristics in all growth phases. Nutrient adequate, but not over-specified, 3 (SFR:0-10-22-37d) or 4 (TAMU 1&2, IRTA:0-10-21-35-42d) phase diets were used. Different levels of corn, wheat, soybean meal, rapeseed and sunflower meal, rice and wheat bran were used to provide enough substrate (i.e. phytate). Oat hulls were also added to provide additional structure and calcium levels were relatively low for the different phases. The setup was 24-26 (TAMU 1&2), 52 (IRTA) and 820 (SFR) Ross 308 birds/pen with stocking density at endpoint of 25-30 (TAMU 1&2), 33 (IRTA) and 39 (SFR) kg/m². TAMU 1 was mixed (50-50 per pen), TAMU 2 all male, IRTA 50-50 with 100% male and 100% female pens and SFR mixed as hatched. In the inorganic P free treatment (IPF), a novel consensus bacterial 6-phytase variant (PhyG) added at 3000 (starter), 2000 (grower), 1000 (finisher 1&2) FTU/kg and with reduced Ca (0.2-0.3% unit). The positive control was formulated with mono-calcium phosphate (MCP), without phytase. In the IRTA trial all treatments and in the SFR trial, one PC and one IPF treatments were with 2000 XU/kg xylanase (-71 resp. -75 kcal/kg ME). The phytate-P level was $> 0.33\%$ through all phases in TAMU1&2, with graded reduction at each phase for IRTA (0.34, 0.33, 0.29, 0.28% in 4 phases) and SFR (0.33, 0.32 and 0.26% in 3 phases). In all 4 studies, IPF treatments maintained or improved performance and bone ash/bone strength vs respective PC in each phase. The final BW (g) in IPF treatment vs PC was: 2984 vs 2770 for TAMU1 ($P < 0.05$), 3140 vs 2994 for TAMU2 ($P < 0.05$), 2811 vs 2704 for IRTA ($P < 0.05$), 2302 vs 2291 across diets with/without xylanase. Overall body weight corrected FCR was consistently lower in IPF treatment vs respective PC ($P < 0.05$), with average of 3.9%

improvement (1.5-6.7%). This corresponds to a reduction of feed cost (including enzyme costs) per kg BWG by average of 4% (2-6.3%). In conclusion, these 4 studies provided strong evidence that an efficient phytase together with proper feed formulation and management enables formulating diets without inorganic P and this can lead to more cost effective and sustainable broiler production.

Key Words: broilers, inorganic phosphor free, sustainability, novel consensus 6 phytase, production benefit

137 The effects of a multi-component protease alone or in combination with a micro-encapsulated blend of organic acids and essential oils on growth performance and gut health of broilers fed low-digestible feed ingredients. Pattaveekan Preesong^{*1}, Glenmer B. Tactacan², Akaradet Seemacharoenstri², Yuwares Ruangpanit¹; ¹*Animal Science, Kasetsart University, Kamphangsaen, Nakhon Pathom, Thailand,* ²*Research and Development, Jefe, Saint-Hyacinthe, Quebec, Canada.*

The fluctuating costs of traditional feed ingredients have limited their use in broiler diets in favor of cheaper raw materials. However, due to their relatively lower digestibility, diets formulated with these raw materials often lead to poor animal performance and gut health. A study was conducted to investigate the effects of a multi-component protease alone or in combination with a micro-encapsulated blend of organic acids and essential oils on the growth performance and gut health of broilers fed low-digestible feed ingredients. A total of 1400-one-day-old Ross 308 male broiler chicks, in a completely randomized design study were assigned to receive 1 of 4 dietary treatments: 1) Positive control (corn-soybean meal-based diet, PC), 2) Negative control (corn-wheat-soybean meal-corn DDGS-based diet, NC), 3) NC + 125 g/t protease (NC+P), 4) NC + 125 g/t protease + 300 g/t micro-encapsulated organic acids and essential oils (NC+P+EOS). Each treatment consisted of 14 replicates with 25 birds per replicate. All chicks were provided feeds and water ad libitum for 35 days. Growth performance, gene expression of jejunal tight junction proteins, and cecal fermentation metabolites were determined at the end of the study. Data were subjected to ANOVA using the PROC GLM procedure of SAS and differences between means were determined using Tukey's honestly significance difference test. At 35 d, body weight gain was similar among treatment groups, but birds fed diets with low-digestible feed ingredients (NC, NC+P, and NC+P+EOS) had significantly increased feed intake ($P < 0.05$) than birds fed the PC diet. Consequently, feed conversion ratio (FCR) was significantly increased ($P < 0.05$) in NC than PC (1.529 vs. 1.466), but was similar to NC+P and NC+P+EOS (1.529 vs. 1.517 and 1.498, respectively). In terms of the gene expression of the tight junction proteins, occludin and claudin-1 genes were upregulated ($P < 0.05$) in birds fed the NC+P and NC+P+EOS, respectively, as compared to PC and NC. Cecal ammonia was significantly increased ($P < 0.05$) in all

diets with low-digestible feed ingredients (NC, NC+P, NC+P+EOS) relative to PC, but cecal putrescine and cadaverine in NC only was significantly increased ($P < 0.05$) as compared to PC, NC+P, and NC+P+EOS. Overall, feeding broiler chickens with diets containing low-digestible feed ingredients negatively affected feed efficiency, intestinal integrity, and concentrations of protein fermentation metabolites in the cecum. On the other hand, the supplementation of a multi-component protease alone or in combination with a microencapsulated blend of organic acids and essential oils alleviated these negative effects and appeared to offer benefits on both animal performance and gut health.

Key Words: Protease, Organic acid, Essential oil, gut health, broilers

138 Evaluating the benefits of a novel multi-component protease supplementation in broiler chickens fed sufficient or low-protein diets. Anhao Wang^{*}, Rob Patterson, Anangelina Archile; *CBS Bio Platforms, Calgary, Alberta, Canada.*

Two studies were conducted to evaluate the effects of dietary supplementation of a novel multi-component protease (Pro) on broiler chicken performance and the apparent total tract digestibility (ATTD). Study 1 was a 28-d feeding trial where 600 newly-hatched male Cobb 500 chicks were randomly assigned to one of six dietary treatments: standard protein control (SP); low protein control (LP); LP with 125 mg/kg Pro (125 Pro); LP with 250 mg/kg Pro (250 Pro); LP with 200 mg/kg single-component protease-1 (SCP-1) and LP with 500 mg/kg single-component protease-2 (SCP-2). The diets were corn-wheat-soybean meal (SBM)-based with SP diets formulated to meet nutrient requirements and had 0.6 - 0.8% higher CP than their LP counterparts. Performance was measured at 12, 21 and 28-d. The ATTD of crude protein (CP), dry matter (DM) and gross energy (GE) were measured at 28-d. Study 2 was a 35-d feeding trial where 400 newly-hatched male Cobb 500 chicks were randomly assigned to one of four dietary treatments: high protein control (HP); SP; SP with 125 mg/kg Pro (125 Pro), and SP with 250 mg/kg Pro (250 Pro). The diets used in study 2 were corn-SBM-based with copra meal and feather meal added as additional protein sources. The HP diets contained 0.6 - 0.8% more CP than their SP counterparts, respectively. Performance was measured at 12, 21 and 35-d. In both studies, each treatment was replicated in 10 identical floor pens with 10 birds/pen. Furthermore, all diets contained phytase at 500 FTU/kg and deployed treatments utilizing a completely random design. In both studies, body weight (g), average daily gain (g/d) and average daily feed intake (g/d) were not affected by treatments at the end of trials ($P > 0.05$). In study 1, protease supplementation reduced feed conversion ratio (FCR) during the 28-d trial period ($P = 0.017$) such that 250 Pro-fed birds had significantly lower FCR than LP-fed birds (1.392 vs 1.423), while 250 Pro-fed birds had higher ($P < 0.05$) ATTD for CP than LP-birds (73.39 vs. 71.80%).

Additionally, ATTD GE was greater ($P = 0.047$) in 250 Pro-fed birds (77.01%) than those fed SCP-1 (76.08%) and SCP-2 (75.92%), respectively. In study 2, Pro supplementation reduced FCR during the entire 35-d feeding period ($P < 0.001$) such that SP birds had significantly higher FCR than 250 Pro-fed birds (1.489 vs 1.469). In both studies, FCR did not differ between 125 and 250 Pro-fed birds ($P > 0.05$). Taken together, supplementing Pro in phytase-containing diets (both nutrient sufficient and low crude protein diets) reduced FCR. The improved FCR observed in multi-component protease-fed birds is likely due to increased nutrient digestibility.

Key Words: multi-component protease, protein density, broiler chicken performance, digestibility

139 Effects of a multi-component protease supplementation on growth performance and carcass yield of Indian River broiler chickens. Mohiuddin Amirul Kabir Chowdhury^{*1}, Sumiya Akter², Subhash Chandra Das², Bapan Dey², Glenmer B. Tactacan¹; ¹Research and Development, Jefe, Saint-Hyacinthe, Quebec, Canada, ²Animal Science, Bangladesh Agricultural University, Mymensingh, Bangladesh.

The use of nutrient matrix values in diet formulation is successfully practiced worldwide to maximize the effect of protease enzymes. An experiment was conducted to evaluate the effects of a dietary multi-component protease on growth performance and carcass characteristics of broiler chickens fed a standard or a reduced diet reformulated considering the recommended nutrient matrix of the supplemented protease with reduced crude protein (CP), amino acids, and metabolizable energy (ME). Standard or positive control diet (PC) was formulated with no supplemental protease containing 23.2 and 21.8% CP, 3000 and 3100 kcal/kg ME, and 1.28 and 1.15% dig. Lys and 0.51 and 0.47% dig. Met for starter and grower diets, respectively. The reduced or negative control diet (NC) contained 22.6 and 21.2% CP, 1.25 and 1.12% dig. Lys, and 0.50 and 0.46% dig. Met., and 2975 and 3075 kcal/kg ME for starter and grower diets, respectively. Three other diets were formulated by supplementing protease to the diets: PC+125 = PC supplemented with 125 ppm protease; NC+125 = NC diet supplemented with 125 ppm protease; and NC+200 = NC diet supplemented with 200 ppm protease. A total of 630 one day old Indian River male broiler chickens were randomly assigned to five treatments with 7 replicates each and 18 birds per replicate. The birds were fed experimental diets for 28 days. Performance data were recorded weekly while carcass yield was determined on day 28. The data were subjected to ANOVA using the PROC GLM procedure of SAS and differences between means were determined using Tukey's honest significance test. Feed intake (FI) was similar among the treatments except in the 2nd wk where protease supplemented groups had higher ($P < 0.05$) FI than those fed the PC diet. Body weight (BW) and body weight gain (BWG) were not different among the treatments at 1st and 2nd wk, however,

at 3rd and 4th wk, broilers supplemented with the protease were heavier ($P < 0.01$) than those fed the PC and NC diets. A similar trend was observed at 1st and 2nd wk on feed conversion ratio (FCR), however, at 3rd and 4th wk, birds supplemented with protease was only better ($P < 0.05$) than birds fed the NC diet. The percentage weights of thigh, breast, and heart in birds fed the NC + 200 diet were greater ($P < 0.05$) as compared to those fed the NC diet. The result indicated that the supplementation of a multi-component protease either on a standard or a reduced diet improved the overall production performance of a commercial broiler chicken. The carcass yield can also be improved by protease supplementation to the cost-effective reduced diet bringing significant economic benefit to the farmers.

Key Words: protease, growth performance, carcass yield, feed cost, broiler

140 Hen response to B-mannanase addition on top to a feed formulated with phytase and xylanase feed enzymes. Sergio R. Fernandez^{*1}, Marco A. Martinez¹, Diego Puron², Raul Santamaria²; ¹Nutritional, Elanco Animal Health, Guadalajara, Jalisco, Mexico, ²Centro de Investigación Animal Aplicada de Yucatan, Merida, Yucatan, Mexico.

Practically, at present day, all poultry feeds are formulated with phytase and xylanase (PX) feed enzymes activities, therefore, the evaluation of feeds without these enzyme activities addition has become irrelevant, however, the presence of the antinutritional factor B-mannan in vegetable feedstuffs, opens the possibility to include a B-mannanase activity in combination with PX in poultry feeds. So, to evaluate the hen response to these activities, 144 30-week-old Bovans White laying hens were randomly assigned to 2 treatments with 6 replicates each, and 12 hens per replicate (3 hen cages with 4 hens/cage). Trial lasted 20 weeks. Treatments were as follows: 1. A corn-SBM feed + 600 FTU/kg *E. coli* phytase + 16,000 BXU/kg *Trichoderma reesei* with 100 kcal/kg formulation matrix value, formulated to fulfil the Bovans White hen nutritional requirements, and 2. As 1 + 52,800 B-mannanase U/kg *Paenibacillus lentus* (Hemicell HT[®]). Data was analysed as a Completely Randomized Design (CRD) with 2 treatments and 6 replicates per treatment. The following variables were not affected ($P > 0.05$) by treatment; egg production, 93.7%, feed intake, 94.5 g/h/d, hen mortality, 0.25%, hen final weight, 1.671 kg, shell strength, 4.566 kg, Haugh units, 95.4, and yolk colour, 12.1 DSM Yolk fan, whereas the following variables were statistically different as follows: feed conversion rate (FCR), as feed intake/egg mass: 1.781 vs 1.720 ($P < 0.03$), cumulative egg mass (CEM), 7.462 vs 7.659 kg/hen (197 g extra/hen, $P < 0.02$), shells stained with hen excreta (EXCR) 6.2% vs 4.4% ($P < 0.04$) for TRT1 and TRT2 respectively. In conclusion, degradation of the antinutritional factor B-mannan, allowed the hen to show better response for FC, CEM, and EXCR, on a feed already with phytase-xylanase activities. Due to the response on FCR, whereas feed formulation cost was

0.9% more expensive for the treatment with B-mannanase on top (0.343 vs 0.346 dollars/kg of feed), egg production cost was statistically the same for both treatments, 0.603 dollars/kg of egg ($P > 0.05$).

Key Words: Hens, B-mannan, B-mannanase, Phytase, Xylanase

141 The efficacy of xylanase and DFM combination in disease challenged floor raised broilers. Yun-mei A. Lin^{*1}, Basheer Nusairat², Rasha Qudsieh¹, Jeng-Jie Wang¹, Nasser Odetallah¹; ¹BioResource International, Durham, North Carolina, United States, ²Department of Animal Production, Jordan University of Science and Technology, Ar-Ramtha, Jordan.

The approach of supplementing poultry feed with a combination of xylanase and direct-fed-microbials (DFMs), along with other management practices, to improve gut health is gaining increased attention as feed-additive alternatives to replace growth promoting antibiotics (AGP). The objective of this study was to evaluate the efficacy of various combinations of different endo-xylanases and multi-strain *Bacillus* spp. products from different commercial sources upon supplementation to corn-soy-based broiler diets based on live performance, gut lesion score, and microbial load of broilers challenged with *Clostridium perfringens* and *Eimeria* Spp. A total of 3,120 Ross 708 mixed-sex broiler chicks were randomly assigned to 1 of 6 dietary treatments with 10 floor pens per treatment, and 52 birds per pen. The 6 treatment were: (PC) standard control diet with no disease challenge; (NC) standard control diet with disease challenged; (NC+A) the NC + xylanase 1 (10 XU/g of feed) + DFM blend 1 ($> 10^5$ CFU/g of feed); (NC+B) the NC + xylanase 1 (10 XU/g of feed) + DFM blend 2 ($> 10^6$ CFU/g of feed); (NC+C) NC diet + xylanase 2 (15 XU/g of feed) + DFM blend 1 ($> 10^5$ CFU/g of feed); (NC+D) NC diet + xylanase 2 (15 XU/g of feed) + DFM blend 2 ($> 10^6$ CFU/g of feed). The xylanases and DFMs are commercial products from different manufactures. The dose tested in this trial was in accordance with the manufacturer's recommended dose. A one-way ANOVA analyses was performed, and the significance was accepted at $P < 0.05$ for all parameters. NC + A and NC + D performed similarly to PC, increased ($P < 0.01$) BW, improved FCR and flock uniformity, and reduced mortality, as well as reduced ($P < 0.01$) lesion score, and microbial load (*E.Coli*, APC, *Salmonella* incidence, and digesta *C. Perfringens*) at 42 days of age compared with NC, while NC+B and NC+ C demonstrated intermediate outcomes. The findings in this study confirm that supplementation of the proper endo-xylanase and multi-strain *Bacillus* spp. combination is an effective technology to improve gut function and gut protection, reduce the pathogen load and environmental contamination, while alleviating the negative effects on growth performance, feed efficiency, and mortality in the presence of a disease challenge scenario such as *Clostridium perfringens* and *Eimeria* Spp. The selection of the correct

xylanase and DFM to use in combination is key to achieve such functions.

Key Words: Xylanase, DFM, lesion score, pathogen load, antibiotic free

142 Diets with varying inclusion rates of phytase and energy levels influence broiler performance from 1 to 42 days of age. Joseph P. Gulizia^{*GS}, Susan M. Bonilla, Jose I. Vargas, Santiago J. Sasia, Leah C. Smith, Wilmer J. Pacheco; *Department of Poultry Science, Auburn University, Auburn, Alabama, United States.*

Phosphorus (P) plays an important role in bone formation and energy metabolism. Inorganic P sources are used in broiler production to fulfill P requirements, but they are predicted to be depleted in less than 50 years. Inorganic P sources can be reduced by utilizing exogenous phytase, which breaks down phytate molecules inherent to plant-based feedstuffs and increase P digestibility. The objective of this study was to evaluate the effects of OptiPhos Plus, which is an intrinsically heat stable form of phytase, with varying inclusion rates and energy levels on broiler performance from 1 to 42 d of age. A total of 1,250 d-old Ross x YPM 708 male broilers were randomly distributed to 50 floor pens assigned to 5 dietary treatments with 10 replicates per treatment. A negative control (NC) diet was formulated to contain -0.20% less Ca and avP at each feeding phase. A positive control (PC^P) was formulated to be sufficient in P with the addition of inorganic P sources. A PC for energy (PC^E) was formulated to contain +100 kcal/kg than the other treatments. Treatments consisted of PC^P, PC^E, NC, NC + 500, and NC + 1500 FTU/kg Optiphos Plus. Feed intake and BW were determined at 14, 28, and 42 d of age and FCR corrected for mortality. Data were analyzed using the GLM procedure of JMP and means were separated by Tukey's HSD with statistical significance considered at $P \leq 0.05$. During the entire trial, broilers fed the NC had the lowest BW compared to the other treatments ($P < 0.05$). Increasing 100 kcal/kg in the PC^E treatment improved FCR by 5 points compared to remaining treatments between d 1 to 42 ($P < 0.05$). Overall, providing phytase at 500 or 1500 FTU/kg in P reduced broiler diets can result in comparable d 42 BW, feed intake, and FCR to PC^P sufficient in P ($P > 0.05$). However, the addition of phytase was not able to improve FCR to the level of the PC^E from 1 to 42 days of age.

Key Words: uncoated phytase, broilers, energy, phosphorus

143 Effects of protease addition on varying levels of CP/AA on performance, egg parameters and apparent amino acid digestibility in 50-70 wk old Hy-Line W36 laying hens. Victoria R. Williams-Hodge¹, Kelley G. Wamsley¹, Kevin Roberson², Pratima Adhikari^{*1}; ¹Poultry Science, Mississippi State University, Starkville, Mississippi, United States, ²CSA Animal Nutrition, Dayton, Ohio, United States.

Supplementing exogenous protease may promote amino

acid (AA) utilization in low crude protein (CP) diets. This study was conducted to determine the effects of reducing CP/AA in diets with or without a commercially available protease on post-peak laying hen performance, egg quality, and apparent ileal digestibility (AID). A 20-week study was conducted using 672 Hy-Line W36 laying hens (50-week-old at the beginning of the study), which were equally and randomly placed into 96 cages as experimental units (raised wire cages). There were 12 replicate cages per treatment with a stocking density of 871.4 cm²/hen. Each replicate cage (7 hens) was fed one of the 8 diets from the 4 AA levels (100, 95, 90, or 85% of breeder recommendation) x 2 protease inclusions (without or with). The adequate (100%) diet was based on corn, soybean meal, and DDGS and formulated based on digestible Lys: digestible AAs (dMet, dThr, dTrp, dTSAA, dIle, and dVal) for 100% of the Hy-Line W36 recommendation. Data were subjected to 2-way ANOVA (PROC GLM procedure, SAS 9.4). There were significant AA level x protease interactions for hen-day egg production (HDEP), percent yolk, and AID for CP, dMet, dThr, dIle, and dVal ($P < 0.05$). For HDEP, 95% without protease was significantly higher than both levels of protease at 85 and 90%, along with 95% with protease ($P=0.0069$). The percent yolk for 100% with and 95% without protease were significantly higher than those fed 90% with and 85% without protease ($P=0.0011$). The AID for CP was significantly higher for 100% without protease than for 95% and 85% with and 90% without protease ($P=0.0026$). However, Met at 100% without protease was significantly higher than for 90 and 85% with and 85% without protease ($P=0.0145$). For both Thr and Ile, 100% without protease had higher AID than 95 and 85% without protease ($P=0.0064$ and 0.0022). Similarly, the AID of Val was higher for 100% without than 90% without protease. The main effect of the AA level significantly affected multiple parameters. As such, egg weight (EW) was higher in hens fed adequate AA level vs. those fed other AA levels ($P=0.0003$). A significant decrease in egg mass was observed when the AA level decreased from 90 to 85% ($P<.0001$). Furthermore, hens fed 85% AA level diets had a significantly higher FCR than those fed all other levels ($P<.0001$), and Haugh units significantly increased as the AA level decreased ($P<.0001$). No interactions or effects were found for feed intake, specific gravity, shell thickness, percent albumen, or total solids. Since no main effect of protease was observed, it may not effectively promote AA utilization in low CP diets for commercial laying hens.

Key Words: amino acids, egg quality, layers, protease, performance

144 Effects on phytase and coccidial vaccine on growth performance, bone mineralization and nutrient digestibility of broilers fed with nutrient-reduced diets. Hanyi Shi*^{GS}, Jinquan Wang, Woo K. Kim; *Poultry Science Department, University of Georgia, Athens, Georgia, United States.*

An experiment was conducted to evaluate effects of phytase

and coccidial vaccine on growth performance, bone mineralization and nutrient digestibility in broilers fed regular diets or nutrient-reduced diets. The experiment was conducted in a 2×4 factorial arrangement with 6 replicates per treatment and 10 birds per replicate. Two main factors were coccidial vaccine and dietary treatments: 1) a positive control (PC; 0.90% Ca and 0.45% avP); 2) a negative control (NC; 0.75% Ca, 0.30% avP, 95% essential amino acid (EAA) and 95% crude protein relative to PC); 3) NC + 500 FTU/kg of phytase; and 4) NC + 1500 FTU/kg of phytase. 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. No interaction effect of phytase and coccidial vaccine on growth performance nor on bone ash or on apparent ileal digestibility (AID) was observed, whereas main effects were observed. Birds fed NC diet showed lower ($P = 0.007$) BWG during d 0-21 compared to PC birds, whereas supplementing 500 or 1500 FTU/kg phytase increased BWG to the same level as PC. During d 0-21, vaccinated birds had lower ($P < 0.001$) FI and ($P = 0.045$) FCR compared to unvaccinated birds. For bone ash, NC diet resulted negative effects on bone volume ($P = 0.021$), fat free dry bone weight ($P = 0.012$), ash weight ($P = 0.005$), ash percentage ($P < 0.001$) and ash concentration ($P = 0.019$) at d 21 compared to PC, whereas supplementing phytase at 500 or 1500 FTU/kg in NC diet was able to improve these bone parameters to the same level as PC; however, vaccination did not have any effect on bone ash. The AID of N was increased ($P < 0.001$) by vaccination. The NC and supplementing phytase at 500 FTU/kg or 1500 FTU/kg groups increased ($P = 0.001$) AID of Ca compared to the PC. The AID of P in NC diet was the same as PC diet, and supplementing phytase at 500 FTU/kg or 1500 FTU/kg improved ($P < 0.001$) AID of P. Additionally, NC had a lower AID of DM compared to PC, and supplementing phytase at 500 FTU/kg or 1500 FTU/kg improved DM digestibility. In conclusion, supplementation of phytase at 500 or 1500 FTU/kg improved growth performance, bone mineralization and nutrient digestibility regardless of vaccination.

Key Words: phytase, coccidial vaccine, nutrient-reduced diet, broiler

145 Nutrient utilization and expression of nutrient transporter and tight junction genes in Eimeria-challenged broilers fed diets with different dietary levels of fiber, protein and enzymes. Yang Lin*^{GS 1}, Oluyinka Olukosi²; ¹*Poultry Science, University of Georgia, Athens, Georgia,* ²*Poultry Science, University of Georgia, Athens, Georgia, United States.*

A total of 360 Cobb 500 male broiler chicks were used in two 21-d experiments to study the possibility of exogenous enzymes helping to recover nutrient utilization losses in Eimeria-challenged broilers by regulating intestinal nutrient transporters and tight junction genes. In Expt. 1, 180 birds were allocated to 6 treatments in a 3×2 factorial

arrangement (3 diets with or without *Eimeria* challenge). Each treatment had 6 replicates with 5 birds per replicate. The 3 diets were based on high fiber-adequate protein (HFAP; 4% ADF, 13.5% NDF, 20.3% crude protein, 2752 kcal/kg ME) basal diet. The basal diet was supplemented with no enzyme, xylanase alone or combined with protease. Experiment 2 had the same experimental design with Expt. 1, but had lower fiber and protein content (LFLP; 3.15% ADF, 8.8% NDF, 17.1% crude protein, 2988 kcal/kg ME) supplemented with no enzyme, protease alone, or combined xylanase. The challenged group were inoculated with a solution containing oocysts of *E. maxima*, *E. tenella*, and *E. acervulina* on day 15. Excreta and ileal digesta were collected on d 21 for nutrient utilization measurement. Jejunal tissue was collected on d 21 for real-time PCR analysis. In both experiments, *Eimeria* challenge decreased ($P < 0.01$) ileal and total tract N utilization as well as AME. Enzyme supplementation had no significant effect on nutrient utilization. In Expt. 1, with HFAP diets, *Eimeria* challenge upregulated ($P < 0.05$) gene expression of claudin 1, the glucose transporters (GLUT1 and SGLT1) and peptide transporter (PepT1); but downregulated ($P < 0.05$) the glucose transporters (GLUT2 and GLUT5), and amino acid (AA) transporters SLC7A2 and solute carrier SLC7AL. On the contrary, the combination of xylanase and protease downregulated ($P < 0.05$) gene expression of claudin 1 and SGLT1. In Expt. 2, in which birds were fed LFLP diets, *Eimeria* challenge upregulated ($P < 0.05$) the expression of claudin 1 and PepT1 but downregulated ($P < 0.05$) the expression of AA transporters rBAT (neutral and basic AA), cationic AA transporter, SLC7A2 and solute carrier SLC7AL whereas enzyme supplementation had no effect on the expression of tested genes. In conclusion, *Eimeria* challenge triggered changes in expression of tight junction and nutrient transporter genes as well as reduced intestinal nutrient utilization capacity irrespective of the diet type. Potential of supplemental enzymes helping to recover the nutrient utilization losses or reversing the gene expression changes were more obvious in birds receiving the HFAP diet.

Key Words: xylanase, protease, broiler, transporters, tight junction

146 The effect of enzymatically-modified canola meal on growth performance, nutrient utilization and intestinal bacterial population of broiler chickens. Yanxing (Stella) Niu*^{GS1}, Anna Rogiewicz¹, Lan Shi¹, Rob Patterson², Bogdan A. Slominski¹; ¹*Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada*, ²*CBS Bio Platforms Inc., Calgary, Alberta, Canada*.

Canola meal (CM) is a valuable source of protein for poultry, despite containing substantial amounts of dietary fibre components, including non-starch polysaccharides (NSP). Supplementation of poultry diets containing CM with enzymes is an effective mean to improve its nutritive value. In our earlier research, the optimal composition of

ingredient-specific carbohydrase to target the NSP of CM was determined in vitro. The most effective blend containing pectinase, xylanase, and invertase activities lead to depolymerization of 47.9% of NSP with the production of bioactive NSP-hydrolysis products and was used to produce enzymatically modified CM (ECM). Compared with CM, ECM contained less NSP, NDF, total dietary fiber, sucrose, oligosaccharides, and phytate P. Moreover, *Lactobacillus spp.* were highly abundant in ECM when compare to CM (~300 fold increase), suggesting that ECM might offer not only the prebiotic benefits to animals but may also serve as a source of probiotic organisms. The objective of this study was to evaluate the effects of replacement of CM with ECM on growth performance, nutrient utilization and intestinal bacterial population in broilers. Four-day-old Ross 308 broiler chickens were assigned to 3 dietary treatments, each consisting of 8 pens of 8 birds each, and were raised under controlled environment for 21 days. Birds were fed the starter (4-14d) and grower (14-21d) Control diets containing 10% and 20% of CM, respectively, and the experimental diets, where ECM replaced 5% for starter and grower diets, or entire 10% of CM for the starter and 20% of CM for the grower diets. The trial was set up as completely randomized design with pen as an unit. All the statistical analysis was conducted by the SAS program (version 9.4, SAS Institute Inc., Cary, NC). Means were separated by Tukey's honestly significant difference. All statements of significance were based on $P < 0.05$. Replacing CM with ECM did not affect growth performance, but it significantly increased overall total tract digestibility of NSP and dietary AMEn, and decreased sialic acids secretion during the grower period. *Lactobacillus spp.* were present in much larger counts ($P < 0.01$) in ileal and cecal digesta of birds fed the ECM diets. The abundance of *E. coli* was significantly lower in the cecal digesta of the high ECM diets compared to the Control. Modification of CM with enzymes significantly changed the composition and structure of CM fiber. Replacing CM with ECM improved nutrient digestibility and increased the presence of probiotic organisms in poultry. Therefore, enzymatically-enhanced CM can improve gut health and could support the antibiotic-free feeding programs in broiler chickens.

Key Words: carbohydrase, canola meal, prebiotic, non-starch polysaccharides, broiler

147 Multi-experiment evaluation of increasing phytase activity from Optiphos® and Optiphos Plus® on 21-day broiler performance and tibia mineralization. Victoria Ayres*^{GS1}, Mark Jackson², Staci Cantley², Samuel J. Rochell³, Cole Crumpacker³, Trevor Lee³, Brooke Bodle³, Wilmer J. Pacheco⁴, Martha Rueda Lastres⁴, Christopher A. Bailey⁵, Kimberly N. Gardner⁵, Tim Boltz¹, Joseph Moritz¹; ¹*Animal and Nutritional Sciences, West Virginia University, Morgantown, West Virginia, United States*, ²*Huvepharma, Inc., Peachtree City, Georgia, United States*, ³*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*, ⁴*Poultry Science, Auburn University,*

Auburn, Alabama, United States,⁵Poultry Science, Texas A&M University, College Station, Texas, United States.

Phytase is used in nearly all broiler diets at varying levels and new commercial sources have been developed in recent years. A series of five experiments at four different universities were conducted to investigate broiler performance metrics and tibia mineralization from two mixer-added, *Escherichia coli*-derived, 6-phytases, Optiphos[®], a coated phytase, and Optiphos Plus[®], a recently developed phytase product selected to be intrinsically heat stable, independent of a coating. Treatments for each experiment included a negative control (NC) and the NC + varying dosages of Optiphos[®] and Optiphos Plus[®] up to 1500 FTU/kg. Experimental diets were fed to male broiler chicks for 21 days. On d 21, broilers were euthanized and tibiae were excised to determine tibia mineralization. Experiments 1-4 utilized pelleted diets while Experiment 5 utilized a mash diet. For each experiment, treatments were arranged by a formulated phytase level and source factorial, using a randomized complete block design. In addition, slope-ratio models were created using data from all experiments to evaluate responses to analyzed phytase levels. Live performance metrics and tibia mineralization responses to phytase activity were increased in all five experiments ($P < 0.05$). Performance and bone mineral metrics continued to improve up to approximately 1,500 FTU/kg in a curvilinear fashion, demonstrating a value of super-dosing, the degree employed being dependent on the product used and the cost-benefit economics of implementation. When comparing non-linear response slopes across all analyzed phytase levels and experiments, the data showed that efficacy of Optiphos Plus[®] was approximately 20% higher than that of Optiphos[®] at similar analyzed levels ($P < 0.05$).

Key Words: Broiler, Phytase Source, Super-Dosing, Meta-Analysis

148 Influence of adaptation length and assay method on metabolizable energy of barley supplemented with or without glucanase. Shravani Veluri*^{GS 1}, Oluyinka Olukosi²; ¹Department of Poultry Science, University of Georgia, Athens, Georgia, United States, ²Poultry Science, University of Georgia, Athens, Georgia, United States.

This study was conducted to investigate the influence of adaptation length (AL) and assay methods; total collection,

(TCM) or index method (IM) on apparent metabolizable energy (AME) and nitrogen corrected AME (AMEn) of barley supplemented with or without glucanase (EZ). Cobb 500 broiler chicks at 0 d were randomly assigned to 12 treatments ($3 \times 2 \times 2$ factorial) with 6 replicates and 3 birds/replicate. The factors were three days of AL (10, 7, or 4 d), two enzyme levels (with or without), and two diet types, wheat-SBM basal diet used to determine AME and AMEn of barley and wheat-barley-SBM test diet (TD) in which barley was added to the RD at 300 g/kg inclusion. Birds on 10, 7, or 4 d AL received experimental diets starting from d 11, 14, and 17 of age, respectively. Total excreta associated with feed intake were collected every 8 hours on d 20 and 21. Diets AME and AMEn assay was done by TCM or IM, and barley AME and AMEn were calculated by the difference method. Barley AME and AMEn data calculated using IM or TCM were analyzed for each method as a 3×2 factorial (3 AL and 2 EZ levels) using Mixed Model procedure of JMP. There was no significant AL \times EZ interaction for AME and AMEn determined by either method. For both methods, AME and AMEn of barley was higher in test diets supplemented with glucanase ($P < 0.001$). For AME and AMEn calculated by TCM, 10 d of adaptation produced higher ($P < 0.001$) AME and AMEn values compared to 7 or 4 d adaptation. For comparison of AME and AMEn determined by both assay methods, the data were analyzed as a $3 \times 2 \times 2$ factorial to determine the influence of AL (10, 7, or 4 d), assay methods (IM or TCM), and EZ (with or without). There was no significant 3-way interaction, but there were significant assay methods \times AL and assay methods \times EZ interactions ($P < 0.001$) for AME and AMEn. AME and AMEn calculated by TCM after 10 d adaptation produced higher ($P < 0.001$) values than 7 or 4 d adaptation, whereas there was no significant effect of AL on AME and AMEn determined by IM. In addition, glucanase supplementation improved AME and AMEn of barley assayed by TCM, but not IM. AME ($P < 0.001$) and AMEn ($P < 0.001$) of barley was higher when determined by TCM compared with IM. In conclusion, feeding barley for 10 d rather than 7 or 4 d gave higher AME and AMEn with or without enzymes, and the effect of AL and enzyme supplementation was more pronounced in TCM compared with IM.

Key Words: barley, glucanase, adaptation length, index method, total collection method

Metabolism and Nutrition: Feed Additives

149 Remediation of hydrogen sulfide emissions from poultry excreta by dietary supplementation of a proprietary microbial product. Sam Shen¹, Hector Leyva-Jimenez*¹, Katherine McCormick¹, Derek Haag¹, Beth Galbraith²; ¹United Animal Health, Inc., Sheridan, Indiana, United States, ²Microbial Discovery Group, Franklin, Wisconsin, United States.

Management of offensive odors from intensive poultry production is a requirement for many poultry companies, particularly in densely populated regions. Hydrogen sulfide (H₂S) is one characteristically noxious gas yet sparse research is available on its production or emission control. Detrimental impacts on birds including gut inflammation and depressed performance have been reported when birds are exposed to H₂S for prolonged periods at relatively high concentrations. To reduce odors, multifaceted approaches can be applied, ranging from farm engineering to feed manipulation. Feeding direct-fed microbial (DFM) is one strategy with the potential to reduce odor. The current study used a proprietary *Bacillus* formulation, Novela® (United™ Animal Health, Sheridan, IN), to address this issue. It was hypothesized that *Bacillus* strains would outcompete some naturally present but undesired microbes in the intestine and in the excreta, therefore reducing H₂S formation and emission. The present study employed two treatments, an untreated control and a DFM supplemented group distributed in a complete randomized block design. Male by-product broiler chicks were raised in an environmentally controlled facility equipped with stainless-steel battery units. All birds were fed a typical corn-soy-DDGS diet and Novela® was supplemented at 7.35x10⁴ CFU/g of complete feed. There were 9 pens per treatment and 6 birds per pen. The effectiveness of Novela® in odor reduction was evaluated by monitoring the H₂S emitted from excreta of grower-age birds. Briefly, pooled representative excreta samples from randomly selected pens were homogenized, conditioned with distilled water, and incubated (24 h) under controlled conditions (25-28°C). Dräger® H₂S diffusion tubes (10-300 ppm) were used as the monitoring tool post-incubation. There were 7 incubation units per treatment. Results were analyzed via 2-tailed independent-samples t-test. Hydrogen sulfide emitted from excreta of the control group reached 129.7±21.9 ppm/100 g of excreta while dietary inclusion of Novela® reduced H₂S to 73.9±27.2 ppm/100 g of excreta, a ~43% reduction (*P* < 0.01). Compared to the control, excreta collected from the Novela® group was observed to have less stickiness during homogenization, potentially due to enhanced microbial fermentation and enzyme activity. The current study demonstrates that a proprietary DFM could beneficially impact the intestinal microbiota to effectively reduce H₂S emission.

Key Words: Hydrogen sulfide, broiler, direct-fed microbial, excreta, odor remediation

150 Evaluating the effects of a synbiotic (probiotic + prebiotic) feed additive on performance of broilers compared to a chemical coccidiostat during a necrotic enteritis challenge. Chasity M. Pender*¹, Shelby Ramirez¹, G. R. Murugesan¹, Brett Lumpkins², Greg F. Mathis²; ¹Biomin America Inc., Overland Park, Kansas, United States, ²Southern Poultry Feed & Research, Inc., Athens, Georgia, United States.

The transition to antibiotic-free broiler production has driven a need for the poultry industry and researchers to seek non-antibiotic solutions for disease challenges including necrotic enteritis. The objective of this study was to evaluate the effects of a synbiotic (probiotic + prebiotic) feed additive on performance of broilers during a necrotic enteritis challenge as compared to a commercially available chemical coccidiostat. A total of 800 day-old Cobb 500 broiler chicks were randomly allocated to one of four treatment groups, which consisted of a non-challenged control, a challenged control, a challenged group supplemented with a chemical coccidiostat (zoalene, 125 ppm), or a challenged group supplemented with PoultryStar® BRO (BIOMIN America, Inc., Overland Park, KS; 500 g/MT). Each group consisted of eight replicate pens with 25 birds per pen. Birds were raised on used litter and on days 19, 20, and 21, 1×10⁸ CFU of *Clostridium perfringens* were administered via the feed. The trial was conducted over a 42-day period. Data were analyzed using the GLIMMIX procedure of SAS with significance reported at *P* < 0.05. The challenge significantly impaired bird performance in the challenged control as seen by a 5.9% reduction in final body weight (*P* < 0.05) and a 9 point increase in overall FCR (*P* < 0.05) compared with the non-challenged control. Final body weight and FCR were significantly improved (*P* < 0.05) in the zoalene and PoultryStar® BRO supplemented groups compared to the challenged control and were similar to each other as well as the non-challenged control. Mortality was significantly improved in the zoalene and PoultryStar® BRO supplemented groups compared to the challenged control (0.0% and 0.5% vs. 4.5%, respectively; *P* < 0.05). Overall, broilers fed PoultryStar® BRO performed similarly to birds provided the chemical coccidiostat zoalene indicating that PoultryStar® BRO may be a valuable tool to combat necrotic enteritis in broilers.

Key Words: necrotic enteritis, vaccine, synbiotic, probiotic, coccidiostat

151 Evaluating the combined effects of a synbiotic (probiotic + prebiotic) feed additive and coccidiosis vaccine on performance of broilers compared to a vaccine alone during a necrotic enteritis challenge. Chasity M. Pender*¹, Shelby Ramirez¹, G. R. Murugesan¹, Brett Lumpkins², Greg F. Mathis²; ¹Biomin America Inc., Overland Park, Kansas, United States, ²Southern Poultry Feed & Research, Inc., Athens, Georgia, United States.

Limitations on use of antibiotics and ionophores have more producers looking towards coccidiosis vaccines to reduce incidence of coccidiosis and necrotic enteritis. Though use of vaccines has been shown to reduce disease incidence, they have also been known to have detrimental effects on performance. The objective of this study was to evaluate the effects of a synbiotic (probiotic + prebiotic) feed additive in combination with a coccidiosis vaccine as compared to vaccine administration alone on performance of broilers during a necrotic enteritis challenge. A total of 800 day-old Cobb 500 chicks were randomly allocated to one of four treatment groups, consisting of a non-challenged control, a challenged control, a challenged group administered a coccidiosis vaccine, or a challenged group administered a coccidiosis vaccine and supplemented with PoultryStar® BRO (500 g/MT). Groups consisted of eight replicate pens with 25 birds per pen. Vaccine was spray administered using the recommended dosage at placement, birds were raised on used litter, and on d19, 20, and 21, 1×10^8 CFU of *Clostridium perfringens* were administered via the feed. The trial was conducted over a 42-day period. On d21, three birds per pen were euthanized and intestinal lesion scoring was conducted. Fresh feces from the litter were collected for analysis of oocysts shedding on d14, 21, 28, and 35. Data were analyzed using GLIMMIX procedure of SAS with significance reported at $P < 0.05$. The vaccine only group had impaired performance as seen by an 11 point increase in overall FCR compared with the non-challenged control ($P < 0.05$), while the FCR of the challenged group was intermediate with a 6 point increase over the non-challenged control ($P > 0.05$). Synbiotic supplementation was able to improve FCR by 7 points compared to the vaccine alone group ($P < 0.05$) and the FCR was similar to the non-challenged control. Body weight was not significantly affected by treatment, though overall there was a numerical increase of 120g in the synbiotic group compared to the vaccine alone group. Lesion scores were increased in the vaccine only group compared with the non-challenged control, while lesion scores of the challenged group were intermediate. Synbiotic supplementation was able to improve lesion scores compared to the vaccine alone group ($P < 0.05$) and were statistically similar to the non-challenged control. Oocyst shedding was observed on d14 and d21 in both groups administered the vaccine, suggesting normal cycling of the vaccine oocysts had occurred. Overall, PoultryStar® BRO may be a valuable tool in the toolbox to help alleviate the negative effects on FCR associated with coccidiosis vaccination during a clostridial challenge.

Key Words: coccidiosis, necrotic enteritis, vaccine, synbiotic, probiotic

152 Probiotics as a potential strategy to mitigate bacterial chondronecrosis with osteomyelitis lameness in broilers. Adnan K. Alrubaye¹, Douglas Rhoads², Amer Hasan^{1, 3}, Khwlah Alharbi¹, Abdulkarim Shwani¹, Sonali Lenaduwe Lokuge¹, Kyle Burks¹, G. Raj Murugesan^{5, 4}, Basharat Syed⁴, Shelby Ramirez^{*5}; ¹*Poultry Science, Poult. Sci. 100 (E-Suppl 1)*

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Bacterial Chondronecrosis with Osteomyelitis (BCO) lameness is a major animal welfare issue that affects the commercial broilers industry. BCO lameness results from mechanical stress leading to bacterial infection of the proximal growth plate of the fast-growing tibia and femur. A variety of bacteria can translocate from the respiratory system and/or the gastrointestinal tract into the bloodstream and eventually colonize bone microfractures leading to necrosis. Consequently, this severe necrotic damage reduces the birds' ability to walk or stand. The objective of this trial was to evaluate PoultryStar® BRO (BIOMIN America, Inc. Overland Park, KS) as a strategy to reduce BCO in broilers. A total of 180 birds were assigned to each of 3 treatments: 1) control, wire-floor (CW; n=2), 2) control, fresh-litter (FL; n=3) and 3) dietary supplementation of PoultryStar® BRO (500 g/MT), fresh-litter (BRO; n=3) with 60 birds per pen. The CW pens were the source of the BCO infection as growth on wire flooring is the gold standard for inducing BCO lameness. The CW pens were nearest the cooling pads to allow airflow to travel from the CW raised birds to the remaining treatment pens arrayed in a random block design relative to the CW pens. The intention of this design was to spread the aerosolized bacteria from the birds raised on CW to birds on fresh-litter as a method to induce BCO lameness. The CW treatment was not considered in the statistical analysis as they were only included as a source of BCO pathogenic bacteria. Lameness was assessed daily from d21 to d56 by evaluating ability to walk. Birds that were clinically lame were euthanized and femur and tibia lesions were recorded. Body weights were collected on d56. The effects of BRO supplementation were analyzed using the GLIMMIX procedure of SAS 9.4 (Cary, NC) with LSMeans for means separation when treatment means were statistically different ($P < 0.05$). Body weight on d56 and overall FCR were not different ($P > 0.05$) between FL and BRO. Overall incidence of lameness was reduced ($P < 0.05$) in BRO birds (35%) compared with FL birds (65%). Femoral head separation (FHS) and tibial head necrosis (THN) bone lesions were reduced ($P < 0.05$) in BRO birds compared with FL birds. Although there were numerical reductions in BRO birds for femoral head transitional degeneration (FHT), femoral head necrosis (FHN), and kinky back (KB) compared to FL birds, these were not statistically different ($P > 0.05$). The CW model used in this trial was successful in inducing BCO in the FL birds. The supplementation of BRO was able to reduce BCO in this trial by 46% compared with FL birds and may be a viable strategy to reduce lameness in broilers.

Key Words: probiotics, lameness

153 Performance of broilers fed diets differing on

soluble arabinoxylan content, with or without stimbiotic supplementation. Gilson A. Gomes*, Xavière Rousseau, Tiago T. dos Santos; *AB Vista, Marlborough, United Kingdom.*

The objective of this trial was to evaluate the effect of dietary NSPs and soluble arabinoxylans (sAX) increase when compared against a corn/SBM diet. A total of 2,184, day-old male Cobb 500 broilers, were assigned to 12 treatments, with 7 pen replicates of 26 birds each. Trial design was a 6x2, consisting of 5 graded levels of sAX (from more sAX [100%M, formulated based on wheat, soybean meal, wheat bran, canola meal and sunflower meal] to less sAX [100%L, formulated based on corn, soybean meal, corn DDGS and rice bran], with the 3 intermediate levels obtained from mixing the 2 basal diets), and a corn-soy based diet that served as a reference, with or without stimbiotic (STB, Signis, AB Vista, Marlborough, UK). Average daily gain (ADG), average daily feed intake (ADFI) and mortality were calculated at the end of every feeding phase. Feed conversion ratio was calculated and corrected by mortality (FCR) and by body weight (bwcFCR), and European Production Efficiency Factor (EPEF) calculated. Data was submitted to two-way ANOVA (except livability which was analyzed by Chi-square) and means separated using Student's T-test. Additionally, linear and quadratic orthogonal contrasts were performed using the treatments with graded levels of sAX, with or without STB. Significance was accepted when $P \leq 0.05$, and trends discussed when $P < 0.10$. There was no effect of treatments on livability throughout the whole trial ($P > 0.10$). Several interactions were seen on performance, with birds presenting different responses to dietary sAX content depending on age and supplementation of STB. Overall, performance was optimized when diets with more sAX content were fed to birds, but initially some detrimental effects were seen without STB supplementation, while at later ages the intermediate levels of sAX delivered the highest body weight improvements. Irrespective of that STB improved the performance of birds and further improved the response to sAX. No interactions were noted on EPEF ($P > 0.10$), and no diet effects either ($P > 0.10$), with STB improving EPEF by 12 points ($P < 0.05$). A trend for interaction was observed on bwcFCR ($P < 0.10$), where birds fed diets supplemented with STB showed a linear improvement of bwcFCR when going from diets 100%L to 100%M ($P < 0.05$), whilst no effects of increasing levels of sAX were seen on birds fed diets devoid of STB ($P > 0.10$). In conclusion, sAX can temporarily be detrimental for bird performance, but overall feeding diets with higher sAX would exert more benefits than not. The use of STB improved performance, showing to be a pivotal strategy to improve dietary fiber utilization.

Key Words: non-starch polysaccharides, soluble fiber, linear contrast, quadratic contrast, performance

154 Effect of stimbiotic supplementation and litter reutilization on broiler chickens. Gemma Gonzalez

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The mode of action of a stimbiotic relies on the provision of xylo-oligosaccharides as an immediate compound for microbiota metabolism and xylanase to hydrolyze dietary arabinoxylans into small oligosaccharides utilized by beneficial bacteria in the gut. A 2 x 3 factorial experiment was used to evaluate the effect of stimbiotic supplementation (Signis, AB Vista) and litter reutilization on performance, short-chain fatty acids (SCFA) and microbial populations in the caeca of broiler chickens. Nine hundred Ross 308 broiler chicks (1-day-old) were allocated to six experimental treatments: 1) control diet on fresh litter (CTR_FRESH); 2) the control diet supplemented with 100 g/ton of stimbiotic on fresh litter (STB_FRESH); 3) control diet on reutilized control litter (CTR_CTR); 4) stimbiotic diet on reutilized control litter (STB_CTR); 5) control diet on reutilized stimbiotic litter (CTR_STB); and 6) stimbiotic diet on reutilized stimbiotic litter (STB_STB). Each treatment had ten replicates of 15 animals. Starter and grower diets, based on wheat and soybean meal, and water were available ad libitum. Body weight gain (BWG), feed intake (FI) and mortality corrected feed conversion ratio (FCR) were measured from 0-35 days. The profile of SCFA and the microbial populations (by % guanidine and cytosine [G+C]) on days 21 and 35 were analyzed. Statistical comparisons were performed using a two-way ANOVA (JMP Pro 15). Litter reutilization did not influence BWG. Birds supplemented with stimbiotic gained 50g more than control birds ($P = 0.082$). A significant interaction ($P < 0.05$) was observed for FI and FCR. CTR_FRESH had the highest FI while CTR_CTR and STB_STB had the lowest intake, being the other treatments intermediate. FCR of CTR_FRESH birds was the highest (1.639) and the supplementation with the stimbiotic (STB_FRESH) reduced this by 9 points ($P < 0.05$). The reutilization of the litter, regardless of the origin, resulted in better FCR compared to fresh litter, however the stimbiotic numerically improved FCR in types of litter. No interactions or litter effects were observed in any of the SCFA concentrations measured at 21 and 35 days of age. Stimbiotic reduced cecal lactic acid on d21 ($P = 0.047$) and increased butyric acid on d35 ($P = 0.036$). The reutilization of the litter did not influence the microbial populations compared to the use of fresh litter at any of the ages measured. Some shifts in the microbial populations were observed on d21 with STB but no effects were observed at 35 days of age. Supplementation of broiler diets with the stimbiotic improved performance, which may be due to a promotion of a more fermentative environment in the gut by the action of very minor bacterial species.

Key Words: stimbiotic, performance, intestinal fermentation, microbiota, broiler

155 Withdrawn.

156 Protected organic acids and essential oils improves the intestinal health of broiler chickens raised under field conditions. Cristiano Bortoluzzi*¹, Ludovic Lahaye¹, Jared Oxford¹, Derek Detzler¹, Cinthia Eyng², Nicolle Barbieri³, Elizabeth Santin¹, Michael Kogut⁴; ¹*Jefo Nutrition, College Station, Texas, United States*, ²*Western State Parana University, Mal Candido Rondon, Brazil*, ³*Population Health, University of Georgia, Athens, Georgia, United States*, ⁴*USDA-ARS, Southern Plains Agricultural Research Center, College Station, Texas, United States*.

The objective of the present study was to evaluate the effect of protected organic acids and essential oils [P(OA+EO)] on the intestinal health of broiler chickens raised under field conditions. The study was conducted on 4 commercial farms. Each farm consisted of 4 barns, 2 barns under a control diet and 2 tested barns supplemented with P(OA+EO), totaling 16 barns (8 control and 8 under P(OA+EO)). The control group was supplemented with antibiotic growth promoters (AGP; BMD (50 g/ton) during starter, grower and finisher 2, and flavomycin (2 g/ton) during finisher 3). The tested group was supplemented with 636, 636, 454, and 454 g/ton of P(OA+EO) during starter, grower, finisher 2 and 3, respectively. Both groups were supplemented with narasin (63 and 72 g/ton, for grower and finisher 2, respectively) and vaccinated against coccidiosis (ADVENT[®]) at the hatchery. A total of 80 birds were necropsied (40/treatment; 20/farm; 5/barn) to collect blood for cytokine array analysis and calprotectin (CALP) concentration, jejunal tissue for *I See Inside*[®] (ISI) analysis, and cecal contents to analyze the microbiota and the frequency of antimicrobial resistant (AMR) genes. The data were submitted to ANOVA ($P < 0.05$) or Kruskal-Wallis' test and the frequency of AMR genes was analyzed by Chi-Square test ($P < 0.05$). It was observed that the supplementation of P(OA+EO) reduced ($P < 0.05$) the ISI scores related to intestinal inflammation, such as the infiltration of inflammatory cells in the epithelium and lamina propria and tended ($P = 0.09$) to reduce the serum concentration of CALP. The supplementation of P(OA+EO) reduced the serum concentration of IL-12 ($P = 0.0001$), IL-16 ($P = 0.001$), and Pentraxin-3 ($P = 0.04$). Additionally, P(OA+EO) maintained the cecal microbiota similar to birds receiving AGP, without significant changes in its diversity and composition. The removal of AGP and inclusion of P(OA+EO) reduced ($P < 0.05$) the frequency of five AMR genes, related to gentamicin (3 genes), tetracycline (1 gene), and aminoglycoside (1 gene). Overall, the inclusion of P(OA+EO), and removal of AGP, in the diets of commercially raised broiler chickens beneficially changed the phenotype of the jejunum as shown by the lowered ISI scores which characterizes an improved intestinal health. Furthermore, P(OA+EO) significantly reduced the serum concentration of several inflammatory biomarkers, while maintaining the diversity and composition of the cecal microbiota similar to AGP fed chickens and reducing the prevalence of AMR genes. It can be concluded that P(OA+EO) improved the intestinal

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mucosa health, maintaining the balance of the microbiota and reducing AMR genes, which provides practical advantages for broilers raised without antibiotics.

Key Words: antimicrobial resistance genes, biomarkers, broiler, essential oils, organic acids

157 Assessing the efficacy of bacteriophage therapy to reduce *Salmonella* colonisation in broiler chickens. Anisha M. Thanki*¹, Steve Hooton¹, Robert Atterbury², Natasha Whenham³, Mike Salter⁴, Mike Bedford³, Helen Masey O'Neill⁴, Martha R. Clokie¹; ¹*Genetics and genome biology, University of Leicester, Leicester, Leicestershire, United Kingdom*, ²*School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Leicestershire, United Kingdom*, ³*Ab Vista, Marlborough, United Kingdom*, ⁴*Ab Agri, Peterborough, United Kingdom*.

Objective: Bacteriophages (phages) are natural viruses of bacteria and can be used as an alternative to antibiotics to treat bacterial infections. The objective of this study was to determine if a phage cocktail delivered in feed is able to reduce *Salmonella* colonisation in experimentally-challenged chickens. **Experimental design:** In this study 672 Ross 308 male broilers were used, and the trial was conducted at Drayton Animal Health, UK. Chickens were divided into six treatment groups and each group included 112 chickens. The treatment groups were T1 (control birds); T2 (birds fed the phage diet at a dose of 10^6 PFU/g); T3 (challenged birds); T4 (birds fed the phage diet at a dose of 10^5 PFU/g and challenged); T5 (birds fed the phage diet at a dose of 10^6 PFU/g and challenged); and T6 (birds fed the phage diet at a dose of 10^7 PFU/g and challenged). The phage cocktail was mixed with starter (0-13 days), grower (14-27) and finisher (28-42) diets. Chickens were challenged via oral gavage on day 4 with *S. Typhimurium* strain 4/74 at a dose of 5×10^6 CFU/per bird. Pooled faecal samples from each pen were collected on days 6, 7, 8, 9, 10, 14, 28 and 42 (last day of the trial) to determine *Salmonella* and phage counts. **Methods and materials:** Xylose Lysine Deoxycholate agar media (Sigma) was used for enumeration of *S. Typhimurium* from faecal sample and lysogeny broth media was used for phage counts. **Statement of statistical analysis:** Differences in log transformation were compared between treatment groups. **Results:** In faecal samples collected on days 6, 7 and 8 the *Salmonella* counts were not significantly different in the challenged treatment groups. On day 9 the average faecal *Salmonella* counts from chickens in T6 were 6.33×10^4 CFU/g, which was significantly lower than the counts from group T3 (8.40×10^5 CFU/g) ($p < 0.01$). Furthermore, *Salmonella* was only isolated from 4/16 pens in T6 versus 12/16 pens in treatment group T3. Further significant reductions in *Salmonella* counts were observed in phage treated groups T4, T5 and T6 ($\sim 2.4 \times 10^2$ CFU/g) versus T3 ($\sim 6 \times 10^4$ CFU/g) in faecal samples collected on day 28 ($p < 0.01$). By day 42 no *Salmonella* was detected in the faecal samples collected from group T4 given the lowest phage dose, and in groups T5 and T6 *Salmonella* was only

isolated from 3/16 and 2/16 pens respectively. In comparison *Salmonella* was isolated from 7/16 pens in T3. **Conclusions:** We showed delivering phages via feed was effective at reducing *Salmonella* colonisation in chickens. The lowest phage dose was the most effective and reduced *Salmonella* counts to below detection limits by the end of the trial. Our study highlights phages offer a promising tool against *Salmonella* infections in poultry.

Key Words: Salmonella, poultry, bacteriophages, phages, antimicrobials

158 Effects of guanidinoacetic acid (GAA) on growth and body composition in broilers. Chongxiao (Sean) Chen*¹, Dima L. White², Yuguo H. Tompkins², John E. Thomson³, Ulrike Braun⁴, Woo K. Kim²; ¹*Prestage Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, United States*, ²*Poultry Science, University of Georgia, Athens, Georgia, United States*, ³*AlzChem LLC, Canton, Georgia, United States*, ⁴*AlzChem Trostberg GmbH, Trostberg, Germany*.

Creatine is an essential biomolecule for the proper function of energy transfer and supply. It is known to support muscle growth and strength, shifting whole body composition toward leaner and less fat tissue. Creatine may also benefit bone strength as a result of a more lean body mass. Guanidinoacetic acid (GAA) is the direct endogenous precursor of creatine and, as a feed additive, is an effective source of creatine. The current study evaluated the dietary supplementation of GAA (Creamino[®]) on growth performance, body composition, bone strength, and gait score in broilers. A total of 648 1-day-old male parent stock Cobb 500 chicks were randomly allocated (12 replicates x 18 birds) to three dietary treatments: control (0%), 0.06%, and 0.12% GAA added to basal Corn/SBM diets formulated in four phases (starter (Str), d 0-14; grower (Grw), d 15-28; finisher1 (F1), d 29-42; finisher2 (F2), d 43-56). Birds were raised in floor pens (1.78 m², wood shavings). Feed intake (FI), body weight (BW), and mortality were recorded at the end of each phase. Gait scoring was performed at d 54. At d 56, creatine concentration was measured in breast and leg muscles. The whole-bird composition was measured by dual-energy x-ray absorptiometry (DEXA). Tibias were subjected to breaking strength test and bone ash analysis. Data were analyzed by one-way ANOVA with Duncan's Multiple Range Test for mean separation and $P \leq 0.05$ as the threshold of significance. Treatment effects on growth performance differed only at the end of F1, where BWG for the 0.12 % GAA group was higher (+53 g) than control ($P = 0.015$). Cumulative FCR was consistently lower than control for 0.06% GAA at the end of Grw (0.03 % points) and for 0.12 % GAA at the end of Grw, F1 and F2 though differences did not attain significance. GAA increased creatine concentration in breast muscle ($p=0.011$), while leg muscle creatine was numerically increased. In both muscles, 0.06 % GAA was as effective as 0.12 % GAA. Creatine was higher in the breast than in the leg muscle ($p<0.0001$). For

the whole bird composition, the DEXA results confirmed the hypothesis: 0.12% GAA in the diet decreased the fat% (17.83 % vs 19.38 % in control) but increased lean% (82.18 % vs 80.61% in control). There was no difference observed in whole-body bone mineral density, bone mineral content, or strength assessment. However, the gait scoring analysis showed adding 0.06% of GAA in the diets increased the gait score 0 ratios and decreased the Gait score 1 ratio compared to the other treatments. In summary, adding 0.06% or 0.12% of GAA to the broiler diet could improve growth performance and muscle development. Furthermore, 0.06% GAA could alleviate lameness in heavy broilers.

Key Words: guanidinoacetic acid, creatine, performance, bone quality, gait score

159 Metabolomics shows that guanidinoacetic acid improves breast weights without aggravating breast muscle myopathies through changes in energy metabolism. Martina Klünemann*, Juliano C. de Paula Dorigam; *Animal Nutrition Research, Evonik Operations GmbH, Hanau, Germany*.

Modern breeds of broilers have been heavily selected for high growth rate and breast-meat yield, which has contributed to the onset of Breast muscle myopathies (BMM). A high BMM incidence of ~85% is common in broiler production and nutritional strategies have been used to reduce this problem. It has been reported that while guanidinoacetic acid (GAA) supplementation commonly improves growth performance¹⁻³ it is also sometimes associated with an improvement of BMMs². To test whether GAA affects BMMs we supplemented 0.06% GAA to very low crude protein diets in broilers with high breast meat yield genetics. The low CP diets might reduce BMM incidence to a level where both directions (decrease and increase) can be investigated as otherwise an incidence of 85% only leaves room for improvements but not deteriorations. We tested 340 male Ross 708 broilers with 17 birds/pen and 10 pens per treatment for 49 days. Broilers were fed a corn-soy diet with crude protein levels ~2.5% below the specification in Ross 708 guidelines but with all AA requirements fulfilled. Pen body weights and feed were weighed on day 14, day 25, day 39 and day 49. At final day birds were slaughtered and weights and yields of commercial cuts and BMM scores were determined. Additionally, one bird/pen at day 22 was sacrificed to take blood plasma samples from the right brachial vein for metabolomics. Performance data were subjected to one-way ANOVA and t-test at 5% probability, while score data were analyzed using chi-square test in R. We found that on average GAA fed birds gained 91g more weight ($p < 0.05$) and had 27g heavier breasts ($p < 0.05$). As expected, we found that heavier breasts had a higher BMM incidence ($p < 0.01$). However, breasts with same BMM score were significantly heavier in GAA fed birds ($p < 0.05$) while GAA did not negatively affect proportion of BMM scores ($p > 0.05$). Metabolomics of blood plasma showed no strong systemic effect of GAA when evaluated using Principal

Component Analysis. Still, 4 out of 629 measured plasma metabolites change in response to GAA treatment: GAA, acetylcarnitine, methyl imidazolelactate and methyl thioadenosine ($p < 0.01$). We conclude that GAA increased number of heavy breasts without increasing BMM scores. We suggest that GAA causes these results through increased overall energy metabolism, increased methionine salvage metabolism and reduced protein degradation while not disturbing healthy normal metabolism.

Key Words: guanidinoacetic acid, breast meat myopathy, low crude protein diet, metabolomics, energy metabolism

160 Muscle creatine content and severity of woody breast in male broilers fed varying levels of guanidinoacetic acid. John E. Thomson^{*1}, Randy D. Mitchell², Ulrike Braun³; ¹*Animal Nutrition, AlzChem LLC, Alpharetta, Georgia, United States*, ²*Technical Services, Perdue Foods LLC, Salisbury, Maryland, United States*, ³*Animal Nutrition, AlzChem GmbH, Trostberg, Germany*.

The objective of this trial was to explore the effects of guanidinoacetic acid (GAA) supplementation on performance, meat quality and muscle creatine (CRT) and creatinine (CRN) in broilers. Day-old male, Ross 708 chicks were allocated 46/pen to 30 pens blocked on location. Ten pens were randomly assigned to each of three treatments (T1, control (corn/SBM); T2, as T1 + 0.06% GAA; T3, as T1 + 0.06% GAA in Starter (Str) and Grower (Gro) and as T1 + 0.08% GAA in Finisher I (FI) and Finisher II (FII)). Feeding phases were Str, d1 – 13; Gro, d14 – 27; FI, d28 – 41; and FII, d42 – 53. Feed and water were available ad libitum. Feed intake and live weight were recorded on d27, 41 and 53. After processing on d53, six birds/pen were randomly selected for breast meat yield and CRT and CRN analysis; a sample of breasts (30, 46 and 33 in T1, T2 and T3, respectively) were evaluated for Woody Breast (WB). Performance data were analyzed as a linear mixed model with Pen within Block as a random effect. Breast meat quality effects were compared by Fisher's Exact Test. Muscle CRT and CRN versus WB and treatment were analyzed as one-way ANOVA. Day 27 gain and mortality were not different between treatments ($p = 0.218$, $p = 0.572$). Treatment effect was significant for d27 FCR (T1, 1.378; T2, 1.355; T3, 1.324 kg/kg; $p = 0.043$); however, mortality adjusted FCR was not significant ($p = 0.141$). Because of a mixing error, FCR differences were not carried on to d53. Analyzed diet protein content in T1 was 1.5 and 2.5 % (absolute) higher than T2 and T3 in FI and FII. Final body weights did not differ by treatment. Mean live weight of birds with WB = Hard was 304 g higher than the average of birds rated Semi-Hard or Normal ($p = 0.001$). WB rated as "Normal", "Semi-Hard" and "Hard" was not different between treatments ($p = 0.161$); however, T3 and T2 had 5 to 9% lower percentage of Hard muscle than T1 (20.0, 10.9, and 15.2% in T1, T2 and T3, respectively), suggesting a tendency towards muscle protection from GAA. Breast meat CRT was lower for T1 (4580 mg/kg)

than T2 (4855 mg/kg) ($p = 0.042$) and greater for T3 (5197 mg/kg) than T2 ($p = 0.006$). Breast CRN was low and marginally affected by treatment (12.86, 14.89 and 15.84 mg/kg in T1, T2 and T3, respectively). Hard breast meat had lower CRT (4318 mg/kg) than Semi-Hard (4826 mg/kg) ($p = 0.002$) or Normal (5054 mg/kg; $p < 0.001$). A similar trend was observed for breast CRN with 12.03, 14.54 and 15.26 mg/kg for Hard, Semi-Hard and Normal ($p = 0.006$ for Normal vs Hard). Increasing severity of WB was associated with higher live weight and lower muscle CRT content. GAA supplementation raised muscle CRT content and resulted in a numerically lower proportion of breast meat classified as Hard.

Key Words: guanidinoacetic acid, creatine, myopathy, creatinine, GAA

161 Effects of glucosamine-derived caramels on performance and bone health of broilers. Emanuele C. Goes*, Mirko Betti, Doug Korver; *Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada*.

The rapid growth predisposes broilers to locomotor problems due to excessive stress on immature cartilage. This can reduce broiler welfare and performance, causing economic losses for the industry. This study aimed to evaluate the potential of glucosamine-derived caramels to prevent tibial and femoral head lesions and enhance the performance of broiler chickens from 0 to 42 d. A total of 1,120 broilers were randomly assigned to 14 treatments with 8 replicate cages of 10 birds each. The treatments were: Control (C; commercial-type diet), C + Glucosamine (GlcN; no caramelization), and 4 glucosamine-derived caramels: Light Caramel (LC; produced at 50°C), Brown Caramel (BC; produced at 90°C), LC+fructose, and BC+fructose; each product was included at 0.08, 0.16 or 0.24% of the diet. Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were measured at 10, 25, and 38 d of age. Tibial and femoral head gross lesion scores were evaluated at 42 d. Parametric data were analyzed by ANOVA with means compared by Tukey's test at $P < 0.05$, and non-parametric data were analyzed by Kruskal-Wallis. At 10 d of age, birds fed LC at 0.24% had greater weight (232.82 ± 5.26 g), BWG (19.34 ± 0.55 g), and lower FCR (1.31 ± 0.04 g) than those fed LC+fructose (195.79 ± 19.23 g; 15.78 ± 1.93 g and 1.51 ± 0.16 g, respectively), but was similar to the other treatments, including C. At 25 d of age, LC at 0.16% resulted in greater weight (979.90 ± 43.39 g; $P = 0.02$) than LC+fructose at 0.08% (828.37 ± 105.20 g) but was similar to the other treatments. For the entire period (0 to 38 d), birds fed LC at 0.16% were heavier at 38d ($2,043.5 \pm 129.3$ g; $P = 0.04$) than treatments LC+fructose at 0.08% ($1,797.3 \pm 146.5$ g), LC at 0.08% ($1,885.1 \pm 140.6$ g), BC+fructose at 0.16% ($1,823.1 \pm 147.5$ g) and BC at 0.16% ($1,823.0 \pm 188.6$ g) but was similar to the other treatments, including C. There were no treatment effects on the tibial head lesion scores. The femoral head lesion scores tended to be significant ($P =$

0.07), with LC included at 0.08 responsible for the highest frequency of birds with healthy femurs (87.5% with score 1) or with minimal lesions (12% with score 2), while the C group had 75% of the birds with healthy femurs and 25% with femoral head transitional degeneration (scores 3 and 4). Light glucosamine-derived caramels might be part of a strategy to enhance performance and bone health in broilers; however, more analyses and further studies are required to understand better the mechanism of action.

Key Words: Broiler chickens, glucosamine caramels, locomotor problems, performance, welfare

162 The effect of a dacitic tuff breccia (Azomite®) in corn, soybean, and DDGS based diets that vary in inorganic phosphate source on pellet mill production rate and pellet quality. Tim Boltz*¹, Jon Ferrel⁴, Kristina M. Bowen², Kari L. Harding⁵, Victoria Ayres³, Joe Moritz¹; ¹West Virginia University, Morgantown, West Virginia, United States, ²Nutritional and Food Sciences, West Virginia University, Morgantown, West Virginia, United States, ³Animal and Nutritional Sciences, West Virginia University, Morgantown, West Virginia, United States, ⁴Azomite Mineral Products, Inc., Nephi, Utah, United States, ⁵North Carolina State University, Raleigh, North Carolina, United States.

Feed mill pellet production rate is of great importance to integrated meat bird production. Ingredients such as dried distillers grains with solubles (DDGS), inorganic phosphate source (IPS), and fat influence production rate and feed quality due to their composition. The naturally abrasive properties of an IPS may scour the pellet die and affect feed production rate. The objective of the study was to evaluate the effect of a dacitic tuff breccia (Azomite®) inclusion in a corn, soybean meal, DDGS based diet with either 1.70 % dicalcium (DCP) or 1.75% tricalcium (TCP) on pellet mill production rate, hot pellet temperature, and pellet quality. The assessment included four experimental treatments in a two (DCP or TCP) x two (Azomite (AZM) or no AZM) factorial Latin Square Design across four manufacturing days with complete blocks in each day. Data were analyzed in a mixed-model analysis using the GLIMMIX procedure of SAS with alpha set at $P \leq 0.05$ and a trend defined at $P \leq 0.10$. Average temperature and humidity were 16C and 67%, respectively. There was a six percent increase in production rate when AZM was added to DCP diets (0.99 versus 1.05 MT/hr; $P < 0.001$) and an eight percent increase in production rate when AZM was added to TCP diets (1.10 versus 1.19 MT/hr; $P < 0.001$), demonstrating a significant IPS by AZM interaction. The increased production rate that favored AZM and TCP diets may be associated with an additive effect of pellet die scouring. IPS and AZM interacted to affect pellet quality ($P < 0.021$) demonstrating that increased production rate decreased pellet quality; however, the greatest amount of the observed change (three percent), would likely have little effect on bird performance based on past research.

Key Words: feed manufacture, tuff breccia, production
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rate, phosphate source, throughput

163 Antimicrobial effects against *Salmonella typhimurium* and protein precipitation capacity of tannic acid in an *in vitro* chicken gut pH model and in simulated feed pelleting temperature. Janghan Choi*, Sudhir Yadav, Sasikala Vaddu, Harshavardhan Thippareddi, Woo K. Kim; *Department of Poultry Science, University of Georgia, Athens, GA, Georgia.*

This study was conducted to determine minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of tannic acid (TA) against *Salmonella typhimurium* nalidixic acid resistant strain (ST^{NR}) and to evaluate protein precipitation capacity and antimicrobial effects of TA in an *in vitro* gastrointestinal tract pH model (stimulated gastric pH: 3.5 to 4.0 and stimulated intestinal pH: 6.5 to 7.0 and 7.0 to 7.5) and after being subjected to simulated feed pelleting temperature (40°C, 50°C, 60°C, 70°C, 80°C, and 90°C). Antimicrobial effects with/without bovine serum albumin (BSA) and protein precipitation capacity at different pH values (2.0 to 8.0) of TA and gallic acid (a major component of TA) were evaluated. The MIC and MBC of TA were determined using a micro-dilution method. To compare antimicrobial effects of TA, the agar well diffusion method was used. Protein precipitation capacity of TA and gallic acid was measured using BSA. Unpaired t-test was used to compare antimicrobial effects of TA with/without BSA at different pH points. Two-way ANOVA (Factors: Time and pH) was used in the *in vitro* gastrointestinal tract pH model study, and one-way ANOVA followed by a Dunn's multiple comparison test was used for the simulated pelleting study. Nearly 100% of TA formed complexes with BSA at pH 3.5 to 4.5, and antimicrobial effects of TA were restricted at pH 2.0 to 2.5 and 2.5 to 3.0 with BSA ($P < 0.05$). Gallic acid did not show protein precipitation and antimicrobial effects. The MIC and MBC of TA against ST^{NR} was 40 µg/mL and 700 µg/ml, respectively. Precipitated TA with BSA in simulated gastric pH was released free in the simulated intestinal pH depending upon time and pH factors (Time: $P < 0.05$; pH: $P < 0.05$). Approximately 65% and 97% of TA-BSA complexes were detached at 120 min at pH 6.5 to 7.0 and 7.0 to 7.5. Antimicrobial effects of TA-BSA in the simulated intestinal pH showed statistically similar ($P > 0.05$) antimicrobial effects with pure TA while antimicrobial effects were limited at pH 6.5 to 7.0 compared to pH 7.0 to 7.5 (pH: $P < 0.05$). Stimulated feed pelleting temperature (60°C and 70°C) increased the antimicrobial effects of TA ($P < 0.05$), while protein precipitation capacity was not affected ($P > 0.05$). Therefore, when only pH was considered, TA has potential to exhibit antimicrobial effects in the lower intestine (pH 6 to 7) of broilers, and simulated feed pelleting temperature increased antimicrobial effects of TA. In the future studies, TA as an antimicrobial agent against ST^{NR} should be validated using an *in vivo* chicken model.

Key Words: Tannic acid, tannins, Salmonella

typhimurium, protein precipitation, In vitro model

164 A blend of protected organic acids + essential oils improves intestinal health indicators and reduces intestinal permeability of laying hens raised under commercial conditions. Mariana Lemos de Moraes*¹, Marcia de Souza Vieira¹, Francisco Bertolini Junior³, Guilherme Moreira de Melo Silva², João Marcos Novaes Tavares², Carlos Yassuharu Nakamatsu², Leticia Cury Rocha Veloso Arantes², Elizabeth Santin¹; ¹*Jefo, Saint Hyacinthe, Quebec, Canada*, ²*Grupo Mantiqueira, Primavera do Leste, Brazil*, ³*Safeeds, Toledo, Brazil*.

We investigated the dietary supplementation of Protected Organic Acids + Essential Oils P(OA+EO) in laying hens raised under commercial conditions. A total of 208,000 laying hens at 2-weeks-old were assigned to receive 1 of 2 treatments for 20 weeks: T1: control, T2: P(OA+EO) at 300 g/t (Jefo - fumaric, sorbic, citric and malic acids + thymol, eugenol and vanillin). At weeks 6, 12 and 21, 12 hens/treatment were used for blood sampling and necropsy for the ISI-I See Inside Scoring System Methodology (Kraieski et al., 2017). A completely randomized design consisting of 2 treatments, each with 12 replicates of 1 hen/replicate, was used. To evaluate intestinal permeability, birds were inoculated with FITC-d (fluorescein-isothiocyanate labelled dextran) and blood samples were collected after one and half h. FITC-d is a molecule with high molecular weight that is only detected in the blood when the intestinal mucosa is damaged. The macroscopic ISI alterations were classified to be presented as: macro-total ISI (sum of the scores given for intestine, liver, proventriculus, annex glands, locomotor and respiratory system) and macro-intestinal ISI (sum of the scores given for duodenum, jejunum, ileum and cecum). The histologic ISI alterations were evaluated in the ileum. A low ISI index represents better health status. Data were subjected to one-way ANOVA and T test was used for parametric and Kruskal Wallis for non-parametric data (XLSTAT software). Hens on Jefo P(OA+EO) had lower ($P<0.001$) level of FITC-d recovered in the blood, which can be interpreted as better integrity of the intestinal mucosa and reduced chances of bacterial translocation. They also presented lower ($P<0.05$) total macro- ISI score of alterations at weeks 6 and 12, lower ($P<0.05$) macro-intestinal ISI score at weeks 6 and 21 and, lower histologic-ileum ISI score of alterations at weeks 6 ($P<0.10$), 12, and 21 ($P<0.05$). In addition, at week 21, hens on P(OA+EO) didn't present *Eimeria* oocysts while the control treatment did ($P<0.001$). In conclusion, the blend of Protected Organic Acids + Essential Oils evaluated can be used to improve intestinal and overall health status in commercial laying hens.

Key Words: essential oil, intestinal health, intestinal permeability, laying hen, organic acid

165 The effects of oregano essential oil on layer hen performance and egg quality. Kenneth E. Anderson*¹,

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Oregano (*Origanum vulgare*) essential oil (OEO) contains over 100 compounds with active properties. Four key compounds are carvacrol, thymol, ρ -cymene, and γ -terpinene that have been shown to have antibacterial, anti-inflammatory, and antioxidant properties. Oregano essential oil has been shown to be beneficial for gut health, and subsequent growth and production performance in poultry. The purpose of this research was to investigate the effects of feeding an oregano essential oil throughout the rearing and laying period of Dekalb White laying hens. The study was conducted at the North Carolina Dept. of Agriculture & Consumer Services, Piedmont Research Station in conjunction with North Carolina State University. The pullets were randomly allocated at day-old to one of three groups: CC, basal diet (Control) in rearing and lay periods; CO, basal diet in rearing, and basal in laying plus 300g/t Orego-Stim® provided in the feed (5% OEO on an inert carrier, Anpario plc, UK); and OO, in rearing and laying basal plus 300g/t Orego-Stim® provided in the feed (5% OEO on an inert carrier, Anpario plc, UK). The pullets were moved at 16 weeks and housed indoors in conventional cages of 24 hens per replicate, with 18 replicates per group (432 pullets per group). Space allocation was 80 inches² per hen throughout laying phase from 17 through 73 weeks of age. Feed composition was dictated by the feed consumption and productivity of the hens and water was supplied *Ad libitum*. Egg production and feed consumption were recorded daily then summarized every 28 days, and egg quality was examined the 3rd week of each production period. Statistical analysis was performed with GLM, the means separated (Tukey's t-test) using JMP software (JMP®, Version 14. SAS Institute Inc., Cary, NC, 1989-2019). There were no differences between the treatment groups at 16 weeks of age. Feed consumption on the OO group was higher ($p<0.0001$) than the OC or CC groups. Percent HD production and egg weight in the OO and CO groups were greater ($p<0.001$) than the CC hens. The egg quality nor egg size distribution were affected by OEO in the diet. However when combined with production and egg weight the daily egg mass produced from the OO and OC hens greater than the CC treatment. Hens supplemented with OEO had an overall better HD% egg production and daily egg mass produced compared with control hens even though the OO groups had the lowest body weight at 73 weeks and the CO groups were intermediate with the CC hens being the heaviest. In Conclusion, individual hen reproductive fitness appears to positively influence productivity with more daily egg mass and improved HD% laying performance.

Key Words: white egg layers, egg production, egg quality

166 Evaluation of probiotic and organic chromium on productive performance and energy utilization of laying

breeder hens. Otoniel F. Souza¹, Catarina Stefanello¹, Bárbara Moreira¹, Carine Adams¹, Jessica C. Agilar¹, Elisa François^{*2}, Kelen Zavarize²; ¹*Animal Sciences, Federal University of Santa Maria, Santa Maria, Brazil*, ²*Kemin Industries Inc., Valinhos, SP, Brazil*.

This experiment was conducted to evaluate productive performance and total tract metabolizability of nutrients and energy of laying breeder hens fed corn-soybean meal diets supplemented with probiotic (*Bacillus subtilis* PB6), organic chromium (Cr propionate) or their combination. A total of 32 Rhodes Island Red and 32 White PlymouthRock breeder hens at 55 weeks of age were allocated in individual cages using a completely randomized block design with 16 replicates (8 per strain). After a 28-d period of adaptation, hens were fed 4 experimental feeds in 4 periods of 28 days each, from 55 to 70 wks. Experimental feeds were a Control diet (formulated with corn, soybean meal and wheat bran without additives), Control + Probiotic (supplemented with 500 g/ton of *Bacillus subtilis* PB6; CLOSTAT); Control + Cr (50 g/ton of organic Cr; KemTRACE), and Control + Probiotic + Cr (with both additives). Productive parameters were evaluated per period and in the overall period as: egg production, egg mass, egg loss and dirty eggs as well as feed intake and feed conversion ratio. Excreta moisture was also evaluated per period, and at 70 wk 1% celite was included in the experimental diets to evaluate digestibility. Excreta were collected per cage in the last two days of the experiment and total tract metabolizability of dry matter, nitrogen and energy as well as apparent metabolizable energy (AME) were calculated. Data were subjected to one-way analysis of variance using the MIXED procedure of SAS Institute and significance was accepted at $P < 0.05$. Means were compared by Tukey test. No effect was observed between the hens' strain, which was previously blocked with the experimental design, and treatment was considered as independent variable. Treatments did not affect daily feed intake, egg loss and dirty eggs ($P > 0.05$); however hens fed Control + Probiotic or Control + Probiotic + Cr had higher ($P < 0.05$) egg production and egg mass from 63 to 66 wk, 67 to 70 wk and in the overall period. From 59 to 62 wk and 63 to 66 wk, hens fed Control + Probiotic + Cr had decreased ($P < 0.05$) excreta moisture than hens the Control; however, from 67 to 70 wk, no difference was observed in excreta moisture. Hens fed Control + Cr had higher AME compared to hens fed the Control ($P < 0.05$). In conclusion, diets supplemented with probiotic, organic chromium or their combination resulted in improved productive performance, decreased excreta moisture and increased energy utilization of laying breeder hens from 55 to 70 weeks of age.

Key Words: *Bacillus subtilis*, breeder hen, chromium propionate, egg production, excreta moisture

167 No presentation materials submitted.

168 Microencapsulation of ajowan essential oil within alginate: Effects on performance and intestinal

microflora of broilers. Tahereh Mersadi, Maziar Mohiti-Asli*, Hassan Darmani-Kuhi; *Department of Animal Science, University of Guilan, Rasht, Iran (the Islamic Republic of)*.

Essential oils (EOs) are natural volatile products extracted from plants, with antimicrobial properties proven in *in vitro* trials. The biological activity of EOs can be lost due to volatilization or degradation of active components, limiting their commercial application. Microencapsulation is the process of building a functional barrier between the core and wall materials to prevent chemical and physical reactions while preserving the biological, functional, and physicochemical features of the core materials. The objective of the current study was therefore to preserve EO of ajowan (*Trachyspermum ammi*) by microencapsulation in sodium alginate, as well as to assess the effects of different dietary doses of free and encapsulated forms of this EO on broilers growth performance and the population of certain intestinal bacteria. A total of 320 one-day-old Ross 308 chicks were randomly assigned to 32 floor pens. A completely randomized design with a 2×4 factorial arrangement of treatments consisting of 2 administration forms of EO (free and encapsulated) and 4 inclusion rates of EO to the diet (0, 50, 100 and 150 mg/kg feed) was used in this study. Each treatment had 4 replicates with 10 birds per pen. Birds were fed their respective experimental diets from 1 to 42 d of age. Growth performance traits were measured in this period. On d 42, two birds from each pen were slaughtered and ileal content were collected from the distal ileum for bacterial count enumeration. Results were analyzed using GLM procedure of SAS, followed by Tukey's HSD test when main effects were significant ($P < 0.05$). No interaction between the main effects was observed for any measured traits. Broilers fed encapsulated EO in the diet had a greater ADFI ($P < 0.01$) than those fed free EO in the diets, probably due to the strong odors of free EO which reduces palatability. Broilers fed 100 mg/kg EO of ajowan compare to the control had higher ($P < 0.05$) ADG (61.8 vs. 56.5 g), ADFI (105 vs. 99.7 g) and European production efficiency factor (313.7 vs. 282.5). The ileal count of *E. coli* and coliforms were lower ($P < 0.05$) in broilers fed encapsulated EO than those fed free EO. The lowest ($P < 0.05$) ileal count of *E. coli* and coliforms was observed in broilers fed 150 mg/kg EO of ajowan in the diet, however *Lactobacillus* count was not affected ($P > 0.05$). In conclusion, inclusion of 100 mg/kg EO of ajowan to the broiler's diet can improve growth performance and reduce ileal *E. coli* and coliform count, with the benefits being more evident for the encapsulated essential oil.

Key Words: broiler, essential oil, encapsulation, intestinal microflora, performance

169 The hepatoprotective effects of dietary herbaceous mixture supplementation on liver injury in laying hen through alleviating hepatic lipid deposition and oxidant stress. Yao Zhu*^{GS}, H.Y Zhang, Y.Q. Huang, W Chen; *College of Animal Science and Technology, Henan*

Agricultural University, Zhengzhou, China.

Hepatic steatosis is the most common disease in laying hen, leading to a decrease in egg production and an increase in mortality. Herbs recently are approved to exert a powerful influence on fat metabolism, anti-inflammation and antioxidant. Hence the potential of herbaceous mixture (HM, containing 20% *ocimum basilicum*, 35% *andrographis paniculata*, 10% *silybun marianum*, 20% *azadirachta indica* and 15% glycine) in alleviating liver injury and its mechanisms were studied. A total of 720 394-day-old Hyline laying hen were subjected to 28 days feeding of three experimental diets: (1) Corn-soybean commercial diet (Ctrl), (2) Ctrl+300 mg/kg HM, and (3) Ctrl+1000 mg/kg choline chloride (CC, as positive control diet). Each diet was fed to 8 replicates of 30 birds. The data was analyzed by one-way the analysis of variance (ANOVA) followed by Tukey's multiple comparisons. $P < 0.05$ was identified significant. Results showed that the laying rate was increased ($P < 0.05$) by dietary HM as relative to Ctrl birds. The outcome of hematoxylin-eosin staining indicated that the area and diameter of steatosis vacuoles was notably decreased by HM and CC administration. Dietary HM and CC supplementation reduced hepatic lipid deposition, evidenced by Oil-red. The birds fed HM diets exhibited considerably decreased ($P > 0.05$) lipogenic genes including fatty acid synthase and acetyl-CoA carboxylase, and significantly upregulated ($P < 0.05$) the mRNA expression of hormone-sensitive lipase and lipoprotein lipase. Similarly, dietary HM treatment notably decreased serum low-density lipoprotein cholesterol level and increased high-density lipoprotein cholesterol concentration when compared to Ctrl diet (both $P < 0.05$). Furthermore, dietary with HM or CC significantly enhanced ($P < 0.05$) the activity of superoxide dismutase and glutathione peroxidase in liver. These data revealed that supplementing 300 mg/kg HM efficiently protects liver injury by attenuating lipid deposition and hepatic oxidant stress in laying hens.

Key Words: herbaceous mixture, liver injury, oxidant stress, lipid deposition, laying hen

170 Effects of a direct fed microbial on pullet performance and body composition. Jordyn Samper*^{GS 1}, Miloud Araba², Miguel Ruano², Troy Lohrmann², Mike Persia¹; ¹*Virginia Tech, Blacksburg, Virginia, United States*, ²*Quality Technology International INC, Elgin, Illinois, United States*.

Direct fed microbials (DFM) have been used to improve animal health and performance, with the reduced antibiotic use within the poultry industry. This experiment was conducted to determine the effects of a DFM on pullet body weight, feed intake, Mortality corrected Feed Conversion Ratio (FCRm), and dual-energy x-ray absorptiometry (DXA). The DFM, Q-Biotic™ 1DP, is a *Bacillus subtilis* strain. Two experimental treatments (Control without DFM and Treatment with 300,000 CFU of DFM per g of feed, both of which were confirmed every time feed was mixed) were composed of 12 replicates of 24 Hy-Line W-36 chicks

resulting in 576 chicks to start the experiment. Chicks were fed ad libitum for the first 3 weeks, then provided a controlled amount of feed daily to mimic feed intake according to the Hy-Line performance objectives. At 6 and 12 weeks of age, 6 chicks from each cage were randomly removed to reduce stocking density over time. At 17 weeks of age, 3 birds per cage were euthanized for DXA analysis to determine lean and fatty tissue content. Data were analyzed for ANOVA and repeated measurement analysis using the MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). If significance was detected, LSMEANS were separated using Tukey's honestly significant difference test. DFM-treated pullets reduced feed intake over the length of the experiment. Control pullets consumed 5.63 kg per bird compared to 5.55 kg per bird in DFM-fed pullets ($P \leq 0.01$), but resulted in similar body weights of 1.23 kg per 17 week old pullet, regardless of treatment ($P = 0.98$). The similar body weight with the reduced feed intake resulted in improved FCRm when repeated measures were used ($P = 0.03$) with an overall 17 week FCRm of 4.232 and 4.259 for DFM treated and control fed birds, respectively. Such FCRm improvement was equivalent to \$5/ton feed value at a \$265/ton weighted average feed cost. At 17 weeks of age, no difference in lean or fat tissue were observed ($P > 0.10$). Overall, DFM treatment allowed for more efficient use of the feed consumed, but did not alter the accretion of body tissue over the 17-week pullet growth period.

Key Words: pullet, DFM, performance, body composition, Q-Biotic

171 *In vitro* screening of drinking water acidifiers and their effects on broiler performance, serum biochemistry and intestinal development. Yujun Guo*^{GS}¹, Bo Chen¹, Huaiyong Zhang¹, Y.Q. Huang¹, Peng Li², W Chen¹; ¹*College of Animal Science and Technology, Henan Agricultural University, Zhengzhou City, Henan Province, China*, ²*Novus International, Shanghai, China*.

Combining with the global demand for safe human food and the production of environmentally friendly poultry products, acidifiers are natural organic acids and salts that have received considerable attention as animal-feed additives. In experiment (Expt.) 1, the effects of different concentrations of acidifier on the acidity and hardness of water, as well as the bacteriostasis was firstly evaluated. The results from the one-way analysis of variance (ANOVA) showed that the addition of different doses (0.02%-0.20%) of acidifier significantly reduced the pH of water with comparable hardness of water ($P < 0.01$). Additionally, the bacteriostatic diameters of salmonella and staphylococci in the 0.20% acidifier group were significantly larger than those in the 0.00% group. To define the response of the performance to acidifier (Expt. 2). A total of 600 1-day-old mixed-sex broilers was weighed individually and allocated to five treatment groups with six replicate pens (10 females and 10 males birds/pen) for 42 days, which consisted of the drinking water with acidifier at 0.00%, 0.05%, 0.10%, 0.15% or 0.20%, respectively. The water with 0.15% or

0.20% acidifier resulted in linear and quadratic higher body weight at 42 days, average daily gain and water consumption during 1 to 42 days ($P < 0.05$). Subsequently, Expt. 3 was conducted to evaluate the impact of acidifier on intestine development and tibia mass. With this aim, a total of 250 1-d-old male broilers were weighed individually and allocated to five treatment groups with five replicate pens (10 males/pen) for 42 days, i.e. the basal diet with 0.00%, 0.05%, 0.10%, 0.15%, or 0.20% acidifier in drink water. Data were analyzed using one-way ANOVA, and showed that supplementing 0.10% and 0.20% acidifier to drink water resulted in increased weight, villus high and the ratio of villus high to crypt of duodenum and ileum for 42-day-old broiler. 0.20% acidifier added to drinking water significantly increased tibial calcium content when compared to 0.00% group. In addition, drink water with 0.10-0.20% acidifier decreased the activity of aspartate aminotransferase and alanine aminotransferase, both important indicators for hepatic health. Thus, we conclude that drink water with 0.10-0.20% acidifier promoted the performance, intestinal development, hepatic health of broilers.

Key Words: acidifier, bacteriostasis, intestine development, serum biochemistry, broiler

172 Effects of direct-fed microbial inclusion on production parameters and intestinal microbial population changes in Nicholas Select tom turkeys.

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The dietary inclusion of direct-fed microbials (DFM) is becoming more common as antimicrobial use for growth promotion is no longer approved. The effects of various DFM on poultry performance have been studied; however, limited research has been conducted on the DFM *Bacillus subtilis* C-3102 in turkeys. Therefore, performance and microbial population responses to *Bacillus subtilis* C-3102 were investigated using 720 Nicholas Select tom turkeys. The experiment was conducted between May 2020 and September 2020. Diets were formulated to be nutritionally identical aside from DFM inclusion and were provided in a six-phase feeding program. Diets containing the DFM were formulated to contain 500,000 cfu/g from d1-35 and 300,000 cfu/g of the DFM from d36-133. Digestible lysine was provided at 90% of breed recommendations and all other amino acids were provided at recommended ratios to digestible lysine. Prior to poul placement the barn was neither cleaned nor disinfected. The combination of high ambient temperatures, reduced amino acid plane, and potential challenge vectors from previous flocks were used

to create an environment suitable for potential performance uplift from DFM inclusions. Each diet was provided to 12 replicate pens of 30 turkeys arranged in a randomized complete block design. The experimental unit was one pen of turkeys. Microbial population (MP) changes were evaluated by collecting fecal and litter samples the d prior to each feeding phase change. Composite fecal samples were collected from eight fresh droppings per pen and composite litter samples were collected from nine defined areas per pen. Data was analyzed using the GLM procedure of SAS. Performance results from d1-133 showed birds provided the DFM exhibited a 0.08 improvement in FCR ($P < 0.0001$) and tended to improve BW ($P = 0.0999$) compared to those fed the control. MP analyses of fecal and litter samples indicated higher concentrations of *Bacillus subtilis* C-3102 and total *Bacillus* in DFM fed birds ($P < 0.05$). Fecal MP analysis results from d132 indicated that the ratio of *Lactobacillus* to total anaerobes of birds provided the DFM increased 13.3% compared to those fed the control ($P = 0.0330$). Interestingly, turkeys provided the DFM had lower body weights for the first four phases of this study. When ambient temperatures increased during the last two phases, both BW and the ratio of *Lactobacillus* to total anaerobes increased for DFM fed turkeys. Furthermore, DFM supplementation reduced overall feed cost by \$0.20 per bird. These data indicate that *Bacillus subtilis* C-3102 inclusions improved tom turkey performance, contributed to a beneficial change in intestinal microbial populations, and reduced feed costs.

Key Words: direct-fed microbials, feed efficiency, performance, microbial population changes, feed cost

173 Broiler intestinal health, immunity, and performance responses to an algae-based ingredient vary between basal diet compositions.

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Bioactive algae-based ingredients provide health-promoting compounds and have been primarily investigated in corn/soybean meal-based broiler diets. Wheat is incorporated into rations as a protein and energy source; however, its nutrient profile may result in disparate physiological responses when formulated with algae ingredients. The objective was to evaluate performance, intestinal health, and immune responses to a proprietary algae ingredient (ZIVO Biosciences Inc., Keego Harbor, MI) when wholly substituting wheat for corn in broiler diets. Ross 308 broilers were randomly assigned to 4 treatments consisting of a corn- or wheat-based diet \pm 0.175% algae ingredient formulated to meet breed standards. Birds were housed in 80 floor pens (14/pen) for a 42d study divided into 14d starter, grower, and finisher periods. Body weight was measured weekly and feed disappearance recorded throughout. Intestine, spleen, and

liver samples were collected from 10 birds/treatment at the end of each 14d period for intestinal histomorphology, flow cytometric analysis of splenic immune cell populations, and tissue kinome arrays. A FITC-dextran intestinal permeability assay was performed on d28 and 29 with a 12h feed restriction challenge per replicate day. Data were analyzed with fixed effects of diet type, algae inclusion, and diet × algae interaction using the MIXED procedure (SAS 9.4; significance at $P \leq 0.05$). Wheat-based diets, regardless of algae inclusion, reduced splenic CD1.1⁺ antigen-presenting and CD3⁺ T cells in the first 14d ($P < 0.0001$), reduced serum fluorescence 6.8% on d28/29 ($P = 0.0002$), and improved 42d FCR by 5 points compared to corn-based diets ($P < 0.0001$). Improvements due to the algae ingredient were only observed when included in corn-based diets where it improved 42d FCR by 5 points and increased duodenal and jejunal villus height 10.0 and 15.2% compared to the corn-based control on d28 and 42, respectively ($P = 0.006$ and < 0.0001 , respectively). Feeding corn-based diets + algae ingredient altered splenic T cell subpopulations through 13.4-27.5% reductions in CD3⁺CD8a⁺ cells compared to the corresponding control on d28 and 42 ($P < 0.0001$). Kinome results show a significant innate immune TLR response via MyD88 due to algae inclusion at d14 in both small intestine and liver. By d42, this response shifts to a more growth factor and adaptive immune-oriented response with concurrent signaling changes indicative of lipid metabolism due to algae inclusion. When formulated in corn-based diets, the algae ingredient may improve broiler health and performance, but requires further evaluation in wheat-based diets, emphasizing the need for comparative study of novel feed ingredients in varying basal diet compositions.

Key Words: Broiler, Intestinal Health, Immunity, Algae, Wheat

174 Comparative evaluation of conventional and alternative gut health management programs on growth performance, breast meat attributes, and ceca digesta short chain fatty acid profiles in broiler chickens. Lisa Bean Hodgins*^{GS1}, Mohsen Mohammadigheisar¹, Chaoyue Wang², Shai Barbut², Elijah Kiarie¹; ¹Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, ²Food Science, University of Guelph, Guelph, Ontario, Canada.

Under current Canadian regulations, the majority of commercial broilers are reared according to three gut health management programs: 1) conventional, where some medically important antibiotics (MIA) allowed (CON), 2) raised without MIA (RWMIA), and 3) raised without antibiotics (RWA). However, little is known on comparative growth performance and physiological responses of birds reared on these programs. Therefore we investigated growth performance, breast meat traits, and ceca digesta microbial activity in broilers reared under these programs. Iso-nitrogenous and caloric basal diets were formulated for starter (d 0-14), grower (d 15-28), and

finisher (d 29-42) phases. For medication, CON had bacitracin in all phases and RWMIA had avilamycin in starter and grower phases. For coccidiosis control, CON had nicarbazin in the starter and narasin for the remaining phases while RWMIA had nicarbazin and salinomycin in starter and grower phases and narasin in the finisher. Anticoccidial vaccine (Coccivac) was administered to RWA birds at hatch. RWMIA and RWA had supplemental butyric acid and bacillus based probiotic and RWA was further supplemented with acidifier, yeast and essential oils. 2,304 d old Ross × Ross 708 chicks (equal ♀ and ♂) were placed in 48 floor pens and allocated to treatments (n=8, sex and program combination) based on initial BW. Feed intake (FI) and BW were monitored by phase. On d 28 and 41, 2 birds/pen were necropsied for breast weight, myopathies, and cecal digesta for short chain fatty acids (SCFA). Data were analyzed for the main effects of program, sex, and associated interactions. There was no interaction ($P > 0.05$) between sex and program. Birds on RWMIA and RWA had better ($P < 0.05$) growth and feed conversion (FCR) than CON birds in starter phase. However, there were no program ($P > 0.05$) effects on overall (d 0-42) growth performance. Sex effect ($P < 0.05$) on growth performance was such that ♀ performed better in starter and ♂ in grower and finisher phases and overall (d 0-42). Relative to CON and RWA, RWMIA birds exhibited ($P \leq 0.04$) heavier relative breast weight (g/100g BW) and lower incidences of woody breast (WB) and white striping (WS). Pullets had heavier ($P = 0.01$) breast on d 28 and presented lower ($P \leq 0.03$) incidences of WB and WS on d 41 than cockerels. Treatment effect on SCFA was only noted on d 28 with CON birds exhibiting higher ($P \leq 0.04$) concentration of acetic and propionic acid than RWA birds. Overall growth performance was similar among the programs, however differences in breast meat attributes and transient ceca microbial activity warrant further study. Further investigations should be extended in commercial farm settings to document biological and economic performance.

Key Words: antibiotics, broiler chickens, gut health, growth performance, myopathies

175 Effects of tannic acid on the growth performance, gastrointestinal permeability, antioxidant capacity, oocyst shedding, and nutrient digestibility of broiler chickens infected with *Eimeria maxima*. Janghan Choi*^{GS}, Yuguo H. Tompkins, Po-Yun Teng, Woo K. Kim; ^{GS}Department of Poultry Science, University of Georgia, Athens, Georgia, United States.

The purpose of this study was to investigate the effects of tannic acid (TA) on the growth performance, gastrointestinal barrier integrity, antioxidant capacity, oocyst shedding, and nutrient digestibility of broilers infected with *Eimeria maxima*. A total of 420 one-day-old male Cobb 500 broilers were randomly allocated to 5 treatments with 7 replicates of 12 birds. The five treatments were: 1) Sham-challenged control (SCC; birds fed a control diet and orally gavaged

with PBS); 2) Challenged control (CC; birds fed a control diet and inoculated with 10^4 of *E. maxima* on D 15) 3) TA0.5 (CC + 0.5 mg/kg of TA); 4) TA2.75 (CC + 2.75 mg/kg of TA); and 5) TA5 (CC + 5 mg/kg of TA). On 5 days post-infection (dpi), gastrointestinal permeability was measured by using fluorescein isothiocyanate-dextran (4 kDa). On 6 and 13 dpi, growth performance was measured, and tissue and digesta samples were collected for analyzing intestinal health parameters. Oocyst shedding and fecal moisture were measured from 5 to 11 dpi and 3 to 13 dpi, respectively. The *E. maxima* inoculation effects (SCC v. CC) were compared by unpaired t-test, and the effects of TA in broilers infected with *E. maxima* were compared using the PROC MIXED with a post-hoc analysis (Tukey's test) in a completely randomized design. The TA5 group had lower body weight (BW) compared to those fed CC ($P < 0.05$) during the pre-challenge period. From 0 to 6 dpi, *Eimeria* infection significantly decreased BW and average daily feed intake, and birds fed TA0.5 had significantly higher body weight compared to those fed TA5. From 6 to 13 dpi, birds fed TA5 had significantly lower BW compared to those fed CC ($P < 0.05$). From 7 to 9 dpi, higher oocyst shedding per gram in feces from birds fed CC were observed compared to those fed TA0.5, TA2.75, and TA5 ($P < 0.05$). On 5 dpi, *Eimeria* infection significantly increased gastrointestinal permeability, and the TA2.75 group had significantly lower gastrointestinal permeability compared to the CC group. On 13 dpi, birds fed TA2.75 significantly lowered jejunal total glutathione (GSH) and reduced-GSH (GSSG) compared to birds fed TA0.5. Digestibility of dry matter and ash on 13 dpi were significantly higher in the TA2.75 group compared to the CC group. In summary, 0.5 mg/kg and 2.75 mg/kg of TA decreased gastrointestinal permeability and oocyst shedding and enhanced nutrient digestibility in broiler chickens infected *E. maxima*, whereas 5 mg/kg of tannic acid reduced growth performance and nutrient digestibility. Thus, tannic acid at an appropriate dosage (0.5 to 2.75 mg/kg) can be used to control oocyst shedding and to enhance gut health of broiler chickens infected with *E. maxima*.

Key Words: *Eimeria maxima*, tannic acid, tannins, gut ecosystem, oocyst shedding

176 Dietary grape pomace – Effects on growth performance, intestinal health, blood parameters, and breast muscle myopathies of broiler chickens. Taiwo J. Erinle*^{GS}, Samson Oladokun, Janice MacIsaac, Bruce Rathgeber, Deborah I. Adewole; *Animal Science and Aquaculture, Dalhousie University, Bible Hill, Nova Scotia, Canada.*

The search for alternatives to antibiotics in poultry production is still on-going and has been directed towards investigation of the efficacy of different potential alternatives. However, it is important that the sought alternatives are cost-efficient and have no negative impact on meat quality, for ease of adoption and profit maximization. This study aimed at exploiting an agro-

industrial waste, grape pomace (GP) as an alternative to in-feed antibiotics and assessing the effects on growth, intestinal morphology, ceca microbiota, ceca short-chain fatty acid (SCFA) concentration, blood biochemical parameters, and breast muscle myopathies of broiler chickens. A total of 576 one-day-old Cobb-500 broiler chicks were randomly allotted to three dietary treatments – Negative control (NC, a corn-wheat soybean-based diet), NC + 0.05% bacitracin methylene disalicylate (BMD), and NC + 2.5% grape pomace (GP). Each treatment was assigned to eight replicate pens with 25 birds per pen. Bodyweight (BW), feed intake (FI), and feed conversion ratio (FCR) were determined weekly, and mortality was recorded daily. On d 36, two chickens/pen were euthanized for measuring blood biochemical parameters, ceca SCFA, and ceca microbiota. White striping (WS) and woody breast (WB) incidence were assessed in 4 chickens/pen on d 42. The GP diet increased ($P < 0.05$) average FI throughout the feeding phases compared to the other treatments, but overall FCR was similar. Birds in the GP treatment had higher ($P < 0.05$) villus height (VH) and increased VH: crypt depth ratio in the duodenum and jejunum compared to other treatments. Ceca SCFA concentrations and the incidence of WS and WB were similar across treatments. Plasma Ca and P were significantly higher ($P < 0.05$) in birds fed GP and BMD, compared to the NC. Birds in the GP treatment had significantly reduced ($P < 0.05$) plasma aspartate transaminase than other treatments. Birds receiving GP had a higher ($P < 0.05$) relative abundance of the phylum Bacteroidetes and reduced ($P < 0.05$) Firmicutes compared to other treatments. The relative abundance of *Bacteroides* and *Lactobacillus* genera were higher ($P < 0.05$) among birds fed GP compared to other treatments. Inclusion of 2.5% GP in broiler chicken diets improved gut morphology and modified the cecal bacterial community and blood biochemical profiles with no adverse effect on growth performance and meat quality.

Key Words: grape pomace, broiler chickens, growth performance, gut morphology, ceca microbiota

177 Optimizing cost and growth performance of broiler chickens fed diets with a bio-emulsifier based on lysophospholipids. Vítor Santos Haetinger*^{GS}¹, Catarina Stefanello¹, Yuri Katagiri Dalmoro¹, Guilherme L. Godoy¹, Carine Adams¹, Kelen Zavarize², Elisa François²; ¹*Department of Animal Science, Federal University of Santa Maria, Cachoeira Do Sul, RS, Brazil,* ²*Kemin Industries Inc., Valinhos, São Paulo, Brazil.*

This study was conducted to evaluate the effects of a lysophospholipid-based bio-emulsifier (LPL) added to corn and soybean basal diets on growth performance, carcass yield, and return on investment (ROI) of broiler chickens. A total of 1,400 Cobb 500 male chicks were distributed to 56 floor pens in a completely randomized design. Broilers were fed 8 dietary treatments, with 7 replicates and 25 birds each from d 0 to 43 posthatch. Treatments consisted of 6 degummed soybean oil based diets: positive control (PC1);

PC1 formulated with 500 g/ton LPL (PC1+LPL on top); PC1 formulated with 60 kcal LPL matrix (PC1+LPL60); PC1 formulated with 100 kcal LPL matrix (PC1+LPL100); and two negative controls NC-60 and NC-100 with reductions of 60 and 100 kcal/kg ME, respectively. Two other diets were formulated with acidulated soybean oil: positive control 2 (PC2) and PC2 formulated with 60 kcal LPL matrix (PC2+LPL60). The formulated ME in the PC1 and PC2 were 3,000; 3,100; 3,200 and 3,250 kcal/kg for pre-starter, starter, grower and finisher feeds, respectively. Growth performance was evaluated from d 0 to 42, and on d 43 carcass and abdominal fat yields were calculated. Data were subjected to one-way analysis of variance using the GLM procedure of SAS and when significant means were compared by Tukey test ($P < 0.05$). The ROI was calculated considering the net income difference between PC1+LPL on top vs. PC1; PC1+LPL60 vs. PC1; PC1+LPL100 vs. PC1 or PC2+LPL60 vs. PC2, divided by the investment with the emulsifier product for each treatment until 42 d. There were no effects of soybean oil sources in any parameter ($P > 0.05$). From d 0 to 21 and d 0 to 42, birds fed PC1+LPL on top feed had higher BW gain and lower FCR as well as decreased abdominal fat compared to NC-60 or NC-100 feeds ($P < 0.05$). For the overall period, birds on reformulated diets, PC1+LPL60 and PC1+LPL100, had lower FCR compared to NC-60 and NC-100 feeds, respectively ($P < 0.05$). Considering the gains in revenue of the slaughtered broilers in relation to the investment with LPL in feed, the use of LPL on top had a ROI of 8:1 vs. PC1, whereas the reformulated diet having 60 kcal/kg from LPL demonstrated reduced feed costs and increased ROI (3). In conclusion, a lysophospholipid-based bio-emulsifier improved performance and return on investment of broilers fed standard or reformulated feeds. The LPL product may be added to feed formulations to decrease the usage of costly added dietary fat or to maximize growth performance.

Key Words: biosurfactant, broiler, lysophospholipid, performance

178 The effect of a dacitic tuff breccia (Azomite®) in corn, soybean, and DDGS based diets that vary in inorganic phosphate source on pellet mill energy consumption, live bird performance and amino acid digestibility. Kristina M. Bowen*^{GS 1}, Elizabeth Lynch¹, Tim Boltz¹, Victoria Ayres¹, Jon Ferrel², Joe Moritz¹; ¹Nutritional and Food Sciences, West Virginia University, Morgantown, West Virginia, United States, ²Azomite Mineral Products, Nephi, Utah, United States.

Past research has shown that Azomite (AZM) can increase pellet mill throughput in diets that include inorganic phosphate sources (IPS) of dicalcium phosphate (DCP) or tricalcium phosphate (TCP). We hypothesized that if production rate were held constant then pellet mill energy consumption would decrease for diets that contained AZM due in part to a pellet die scouring effect. This could decrease dietary amino acid exposure to friction and pressure within the pellet die, maintaining amino acid

conformation, and amino acid digestibility. The objective was to determine the effect of AZM (0.25%) in diets with DCP and TCP on pellet mill energy consumption and subsequent live bird performance and amino acid digestibility when fed to broiler chicks for 21 days. Feed was manufactured using a Latin square design with four replications. Post manufacture, three hundred twenty-day-old Hubbard × Ross 708 males (0.038 ± 0.0014 SD) were allocated by weight to 8 replicates per treatment, 10 chicks per raised wire cage, in a randomized complete block design. Dietary treatments were arranged in a 2 x 2 factorial: DCP without AZM, TCP without AZM, DCP with AZM, and TCP with AZM. On d21 ileal contents were collected for amino acid and titanium dioxide analysis. Statistical analysis (Glimmix, SAS 9.4) was performed on the factorial arrangement of treatments and multiple comparison of all treatments with post-hoc by Tukey's. Pellet mill motor load decreased in diets containing TCP compared to DCP (5%; 42.91 vs 44.97, $p < 0.001$, respectively), and when AZM was included (1%; 44.19 vs 43.69, $p < 0.001$). Decreased motor load suggests that feed was subjected to less friction and/or pressure through the die. Digestibility of lysine, cysteine, and isoleucine were affected by the interaction of IPS and AZM inclusion ($P < 0.05$), demonstrating that processing with AZM increased digestibility in DCP diets but not TCP diets. Despite increased amino acid digestibility, AZM in DCP diets increased feed conversion ratio (FCR) by 0.02 (1.284 vs 1.303, $P < 0.05$). However, AZM did not affect FCR in TCP diets (1.294 vs 1.285, $P > 0.05$). Main effect differences for IPS in ADFI ($P < 0.02$) and ADG ($P < 0.03$) favoring DCP occurred. Decreased pellet mill motor load that resulted from AZM inclusion likely reduced feed exposure to friction pressure that preserved amino acid conformation, but the improvement was not translated to growth efficiency.

Key Words: Phosphate, Azomite, Motor load, Amino acid digestibility, Feed conversion ratio

179 Successive delivery of essential oil via *in ovo* and in-water route improves broiler chicken blood biochemical and antioxidant status without altering growth performance. Samson Oladokun*^{GS}, Janice MacIsaac, Bruce Rathgeber, Deborah I. Adewole; *Department of Animal Science and Aquaculture, Dalhousie University, Truro, Nova Scotia, Canada.*

As the poultry industry recedes from the use of antibiotic growth promoters, the need to evaluate possible alternatives and the delivery method that maximizes their effectiveness arises. This study evaluated the effect of an essential oil blend (containing star anise, cinnamon, rosemary, and thyme oil) and its delivery routes on broiler chicken growth performance, blood biochemistry, immune and antioxidant status. A total of 670 Cobb 500 hatching eggs were incubated for 21 days. On d 12 of incubation, viable eggs were randomly allotted to 3 groups: non-injected group, *in ovo* saline group, and *in ovo* essential oil + saline group. On d 18 of incubation, 0.2 mL of essential oil + saline (dilution

ratio 2:1) or saline was injected into the amnion. At hatch, chicks were re-allotted to 6 new treatment groups: in ovo essential oil + in-water essential oil (IWEO), in ovo essential oil (IEO), in ovo saline (IS), in-water essential oil (0.25mL/L water-WEO), in-feed antibiotics (Bacitracin methylene disalicylate-55 mg/kg), and a negative control (NC; corn-wheat-soybean diet) in 8 replicate cages (6 birds/cage) and raised for 28 d. Hatch parameters were assessed on d 0, and body weight (BW), and feed intake (FI) weekly. On d 28, 2 birds/cage were euthanized, and immune organs weighed. Plasma and serum samples were collected for plasma biochemistry, antioxidant activity, and serum immunoglobulins (IgG, IgM) analysis. Data were analyzed in a completely randomized design using the one-way ANOVA model. While the IEO group recorded 4 and 18% reduction ($P < 0.05$) in chick length and hatchability (%), respectively, compared to the NC group at hatch; there was no difference in BW gain, FI, and feed conversion ratio among all groups for the 0-28 d period. The IWEO group showed a tendency ($P = 0.07$) for increased relative weight of liver and plasma calcium level. All in ovo groups had reduced ($P < 0.05$) plasma creatine kinase (CK) levels, while all essential oil groups had reduced ($P < 0.05$) plasma aspartate aminotransferase (AST) levels. The IWEO group recorded 47 and 22% lower levels of CK and AST, respectively than the NC group. No treatment effect was found for serum IgG and IgM levels. The IWEO group total antioxidant capacity (mM uric acid equivalents) was 80% higher ($P < 0.05$) than the NC group. Conclusively, a combination of in ovo and continuous in-water delivery of essential oil offers the potential to improve broiler chicken biochemical and antioxidant status without altering growth performance. This novel delivery strategy presented might portend an effective way to eradicate antibiotics use in the poultry industry.

Key Words: In ovo, essential oil, blood biochemistry, antioxidant, broiler chicken

180 Chitosan oligosaccharides as potential antibiotic replacements in broiler diets. Emanuele C. Goes*^{GS}, Lingyun Chen, Doug Korver; *Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, Alberta, Canada.*

The removal of antibiotic growth promoters (AGP) from animal diets impairs control of necrotic enteritis (NE) and thus broiler chicken performance, resulting in economic losses. Dietary shellfish chitosan oligosaccharides (COS) were screened as potential AGP replacements on performance, NE intestinal lesion scores, and carcass traits of broilers from 0 to 38 d of age. A total of 1,152 birds were randomly distributed across 8 treatments with 8 replicates of 18 birds each. Following a pilot study, treatments were COS 95 kDa at 0.2 and 5 g/kg, COS 180 kDa at 0.2, 2, and 5 g/kg, COS 110 kDa at 5 g/kg of the diet, a positive control (PC; with antibiotic and coccidiostat) and a negative control (NC; without antibiotic and coccidiostat). A natural sub-clinical NE challenge model was applied to all birds, with a

15X oral coccidiosis vaccine dose at 12 d and a 24-hour feed removal at 18 d. Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were measured at 10, 25, and 38 d of age. Intestinal NE lesion scores were measured at 22 and 38 d, and carcass yield at 38 d. Parametric data were analyzed by ANOVA with means compared by Tukey's test at $P < 0.05$, and non-parametric data by Kruskal-Wallis. There were no significant differences between the PC and NC treatment for performance, NE lesions, or carcass traits. After the challenge, animals fed COS 95 kDa at 5 g/kg had a higher BW (904.77 ± 34.33 g) than those fed COS 180 kDa at 5 g/kg (838.99 ± 43.24 g; $P = 0.03$). PC treatment had higher BWG (44.41 ± 1.95 g/day/bird; $P = 0.02$) and lower FCR (1.573 ± 0.07 ; $P = 0.05$) than birds from COS 180 at 5 g/kg group (39.82 ± 2.96 g and 1.703 ± 0.13 , respectively), but were similar to the other treatments. From 0 to 38 d, birds fed COS 95 kDa at 5 g/kg were nearly significantly heavier by 81.6 g than birds from the NC group and by 45.6 g than birds from PC group ($P < 0.08$). No significant treatment effects were found for NE gross lesion scores at 20 d or 40 d. Birds from the NC group had a higher drumstick yield (16.03%; $P = 0.03$) than those fed COS 110 kDa (14.56%), but similar to the other treatments. COS 95 kDa may show some promise to replace AGP; however, complementary analyses and further studies are required to understand the mechanism of action.

Key Words: Antibiotic replacement, broiler chicken, chitosan oligosaccharide, necrotic enteritis, performance

181 Effect of dietary ginger root extract on growth performance and delayed-type hypersensitivity response of broiler chicks. George Dosu*^{GS}, Shengmin Sang, Temitayo Obanla, Yewande Fasina; *North Carolina A&T State University, Greensboro, North Carolina, United States.*

The continued increase in consumer demand for antibiotic-free poultry meat necessitates incessant search for alternative growth promoters. Ginger contains phenolic components that helps to alleviate gastrointestinal diseases and support immune functions and may therefore have potential to replace antibiotics in broiler diets. A 6-week experiment was conducted to evaluate the effect of dietary ginger root extract (GRE) on growth performance. Also, delayed-type hypersensitivity (DTH) response was evaluated as an indicator of cell-mediated immunocompetence. A total of 432 day-old chicks (Ross 708 strain) were obtained from a commercial hatchery, weighed, and randomly assigned to six dietary treatments of varying ginger concentrations as follows: Treatment CON (fed corn-soybean meal (SBM) basal), Treatment MX (fed basal diet containing bacitracin methylene disalicylate (BMD) 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 over the 6-week period. DTH response was evaluated by measuring toe-web swelling as an indicator of T cell-induced DTH response following intradermal injection of Phaseolus vulgaris (kidney bean) agglutinin (PHA-P). Compared to MX (3.32 Kg), BW was lower ($P < 0.05$) for birds in GRE4 (2.92 Kg), but similar ($P > 0.05$) for birds in GRE1 (3.38 Kg), GRE2 (3.27 Kg), and GRE3. FCR was superior for birds given diets supplemented with BMD (1.317) and GRE (1.328 to 1.413; $P < 0.05$) compared to CON birds (1.702). Furthermore, FCR was similar ($P > 0.05$) for MX, GRE1, GRE2, and GRE3 birds. At 24 hours post-PHA-P injection, birds in all GRE-supplemented treatments had DTH response (0.80 mm to 1.02 mm; $P > 0.05$) that was similar to that of MX (0.72 mm). However, CON birds had lower DTH response (0.25 mm; $P < 0.05$) compared to GRE1, GRE2, and GRE3 birds. It was concluded that supplementation of GRE up to 1.5% level of the diet enhanced cell-mediated immunity (DTH response) without compromising bird growth.

Key Words: Ginger root extract, Bacitracin methylene disalicylate, Growth performance, delayed-type hypersensitivity

182 Beneficial effects of *A. niger*-fermented tomato pomace on the growth performance and antioxidant capacity in broiler chickens. Emrah Gungor*^{GS}, Aydin Altop, Guray Erener; *Ondokuz Mayıs University, Samsun, Turkey.*

Finding new natural antioxidants for broiler diets has become more important due to growing customer concerns about synthetic antioxidants. Tomato pomace (TP) is an agricultural by-product having the potential to be a feed additive considering its antioxidative effects. Fermentation has become a useful method for the utilization of agri-industrial residues. Solid-state fermentation refers to the microbial growth in moistened solid substrates. *Aspergillus niger* used as a probiotic in broiler diets can increase the antioxidant capacity of agricultural residues through solid-state fermentation. The effects of TP and fermented tomato pomace (FTP) on the growth performance and antioxidant status in broiler chickens were investigated in the present study. Tomato pomace was fermented with *Aspergillus niger* (ATCC 9142) for seven days. Totally, 140 one-day-old male broiler chickens (Ross 308) were assigned to four treatment groups with five replicates of seven birds per replicate. Birds were fed for a period of 42 days with a soybean-corn-based diet, diets supplemented with 10 g/kg TP, 10 g/kg FTP, and 0.25 g/kg synthetic antioxidants (AO; 5% butylated hydroxytoluene, 1% butylated hydroxyanisole, and 11% ethoxyquin). Data were analyzed in a completely randomized design by one-way ANOVA using SPSS statistical software. Means were separated with Duncan's multiple range test at 0.05 significance level. Dietary FTP and AO improved ($P = 0.025$) the feed conversion ratio, although dietary inclusion of TP did not

affect the growth performance of broiler chickens. Serum glutathione peroxidase ($P = 0.047$) and superoxide dismutase ($P = 0.009$) were increased with dietary supplementation of TP, FTP, and AO. These results showed that FTP has the potential to be used in broiler diets as an alternative to synthetic antioxidants with improving the growth performance and antioxidant capacity of broiler chickens. (This study was supported by Ondokuz Mayıs University, PYO.ZRT.1901.18.008).

Key Words: tomato pomace, natural antioxidant, *Aspergillus niger*, broiler chickens, antioxidant capacity

183 Efficacy of a phytogetic formulation as a replacer of antibiotic growth promoters at improving growth, performance, carcass traits and intestinal morphology in broiler chicken. Naveen S. Murikupudi*^{GS 2, 1, 4}, Anil k. chittithoti³, Susmitha Thulimalli⁴, Aswani Kumar¹, Bhaskar Ganguly⁵, Ravikanth Kotagiri⁵; ¹*Veterinary Biochemistry, Sri Venkateswara Veterinary University, Vijayawada, India,* ²*Animal Biochemistry, NDRI, Karnal, Haryana, India,* ³*Animal Nutrition, Sri Venkateswara veterinary university, Vuayawada, Andhra Pradesh, India,* ⁴*Poultry Science, Sri Venkateswara Veterinary University, Vijayawada, Andhra Pradesh, India,* ⁵*Research & Development, Ayurvet Limited, Baddi, Himachal Pradesh, India.*

The current study aimed to investigate the effects of dietary supplementation of broiler chicken with a phytogetic formulation as a replacer of antibiotic growth promoters on their growth, performance, carcass traits and intestinal morphology. Two hundred and ten (210) one-day-old, straight run Cobb chicks were randomly assigned to either of seven equal groups (T0-T6), each having three replicates, and reared for 42 days in deep-litter system at Institutional Poultry Farm, Gannavaram (16.54° N, 80.80° E) during February to March, 2018 without environmental control (mean ambient temperature 26°C, relative humidity 73%). T0, receiving a standard basal diet without any growth promoting, antimicrobial or anti-coccidial agents, served as the negative control. T1 received a phytogetic growth promoter, Nbiotic™ (Ayurvet Limited, India) at 500 ppm in feed, T2 received Brand A (amoxycillin 50%) at 250 ppm, T3 received Brand B (ciprofloxacin 25%) at 500 ppm, T4 received Brand C (neomycin 10% + doxycycline 10%) at 500 ppm, T5 received Brand D (oxytetracycline 50%) at 500 ppm, and T6 received Brand E (tiamulin 10%) at 500 ppm of feed. Different parameters pertaining to growth, performance, carcass traits and intestinal morphology, including ultrastructural studies, were recorded in the birds and, unless stated otherwise, the statistical significance of the differences between group mean values were ascertained by one-way analysis of variance (ANOVA) at $P < 0.05$. Besides significantly better FCR than groups T0, T3, T5 and T6, and comparable FCR to groups T2 and T4, group T1 showed significantly better weight gain than group T0 and comparable growth to all other groups. Group T1 showed significantly higher values of serum albumin, serum

total protein, height of ileal villi and depth of ileal crypts as compared to the unsupplemented control and the antibiotic-supplemented groups. Group T1 also showed significant improvements in body weight gain, muscle protein content, ileal villous width, jejunal villous height and width, jejunal crypt depth, and blood glucose over the unsupplemented control T0 and most of the antibiotic-supplemented groups. It could be concluded that under the conditions of the study, the phytogetic formulation Nbiotic™ is an efficacious natural growth promoter for broiler chicken and that it could be used to successfully replace antibiotic growth promoters in broiler feeds for improving growth, performance, carcass traits and intestinal morphology.

Key Words: Antibiotics, Growth promoters, Broiler chicken, Nbiotic, Phytogetic

184 Protective efficacy of quercetin supplementation on the dietary-induced fatty liver syndrome in broiler chicken model. Abhishek B. Parmar*^{GS 2.1}, Vipul R. Patel², Jignesh M. Patel³, Umed V. Ramani⁴, Dhruv N. Desai⁵; ¹*Animal Nutrition Research Station, College of Veterinary Science & Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India, Anand, Gujarat, India,* ²*Department of Animal Nutrition, College of Veterinary Science & Animal husbandry, Navsari Agricultural University, Navsari, Gujarat, India, 396450, Gujarat, Navsari, Gujarat, India,* ³*Department of Veterinary Pathology, College of Veterinary Science & Animal husbandry, Navsari Agricultural University, Navsari, 396450. Gujarat., Navsari, Gujarat, India,* ⁴*Department of Animal Biotechnology, College of Veterinary Science & Animal husbandry, Navsari Agricultural University, Navsari, 396450. Gujarat., Navsari, Gujarat, India,* ⁵*Department of Veterinary Microbiology, College of Veterinary Science & Animal husbandry, Navsari Agricultural University, Navsari, 396450.Gujarat., Navsari, Gujarat, India.*

Fatty liver syndrome (FLS) is a type of metabolic disorder related to lipid metabolism that is regulated by various stresses in chickens. Flavonoid compound, quercetin, is having the potential to modulate oxidative stress and lipid metabolism. The present study was designed to investigate the protective effect of quercetin in broiler chickens challenged with high-fat diet (HFD). A total of 192-day old

Vencobb-400 broiler chicks were randomly allocated to four dietary treatments with four replicates (12 birds/replicate) per treatment following a completely randomized block design. Four treatments included corn soya-based basal diet without any supplementation [ME(kcal/kg);3080.56(2-3 weeks), 3185.25(4-6 weeks)], basal diet+quercetin (1g/kg) [ME(kcal/kg);3080.56(2-3 weeks), 3185.25(4-6 weeks)], high-fat diet supplemented with vegetable oil [34g/kg,ME(kcal/kg);3385.45(2-3 weeks), 35g/kg,ME(kcal/kg);3490.31(4-6 weeks)], high-fat diet supplemented with vegetable oil [34g/kg,ME(kcal/kg);3385.45(2-3 weeks), 35g/kg,ME(kcal/kg);3490.31(4-6 weeks)]+ quercetin (1g/kg) in T1, T2, T3, and T4 groups, respectively for the age of 2-3 (starter) and 4-6 (finisher) weeks (35 days duration). Various zootechnical parameters were evaluated daily and weekly basis. Blood was collected on the last day of experiment for the biochemical and antioxidant status assessment. At the end of experiment, birds were ethnized with humane method for carcass and organs studies. The mean of the generated data was subjected to one-way ANOVA and the statistical significance ($P \leq 0.05$) using the package SPSS, version 20.0. The improvement was observed in the growthperformance and feed conversion ratio in broilers with the treatments ($P \leq 0.05$). HFD supplemented group showed increased ($P \leq 0.05$) levels of metabolic stress, explicated by the elevated level ($P \leq 0.05$) of cortisol, MDA, and reducedserum/liver SOD and GPx activity. Moreover, lipotoxicity was found due to the accumulation of fat, and lipid peroxidation caused various injuries to the vital organs like liver and kidney which were manifested by histopathological findings and also upregulated ($P \leq 0.05$) the hepatic inflammatory IL-1 β , IL-6, and TNF- α mRNA expression in T3(HFD) group. HFD combination with quercetin in T4 treatment attenuates the altered serum metabolic markers, lipid peroxidation, and subsequent rise in endogenous enzyme activity ($P \leq 0.05$). In addition, it exhibited lipolytic action by lysis of accumulated fat and ameliorate the pathomorphic alteration in vital organs and downregulation of hepatic IL-1, IL-6, and TNF- α mRNA expression. Thus, it is concluded that quercetin has the potential to protect against the adverse effects induced by high-fat diet consumption in broilers.

Key Words: Broilers, High fat diet, Lipid peroxidation, Pathomorphic, Quercetin

Metabolism and Nutrition: General Nutrition

185 Correlation between data on proximal composition, protein quality indicators, and amino acid profile of commercial samples of soybean meals analyzed by wet chemistry or estimated by NIRS technology. L. Dardabou, H. Kadardar, L. Cámara*, Gonzalo Mateos; *UPM, Madrid, Madrid, Spain.*

A total of 26 commercial soybean meal (SBM) samples were randomly collected in different locations at European crushing plants by specialized personnel and analyzed by wet chemistry and near-infrared spectroscopy (NIRS) for DM, ash, CP, amino acids, ether extract after previous hydrolysis (EEh), crude fiber (CF), neutral detergent fiber (NDF), gross energy, phosphorus (P), and protein quality indicators [PDI, protein dispersibility index; KOH solubility, and TIA, trypsin inhibitor activity]. All wet analyses were performed by the same laboratory using official methods. The NIRS values of the SBM samples were obtained in a second laboratory using their own calibration data. As fed wet chemistry values, independently of the origin of the SBM, varied (% as fed bases) from 85.7 to 91.1 for DM, 6.1 to 6.9 for ash, 44.5, to 49.8 for CP, 1.40 to 3.01 for EEh, 4.1 to 5.2 for CF, 9.8 to 12.9 for NDF, and from 0.58 to 0.69 for total P. When the SBM were sorted by the origin of the beans, the CP as fed wet chemistry values, varied from 45.2 to 46.8 % for ARG, 44.7 to 49.8 % for BRA, and 44.45 to 46.9 % for USA meals. Similarly, the protein quality indicators varied from 8.2 to 19.0 % for PDI, from 59.6 to 85.7 for KOH, and from 1.10 to 2.80 mg/g for TIA. The Pearson correlation (r) analyses using the CORR procedure of SAS (SAS Institute Inc., 1990) were used to study the correlations between the values obtained by the two methods (wet chemistry and NIRS technology), independently of the origin of the SBM effect. Sample of SBM was considered the replicate with a total of 26 samples (USA = 8, BRA = 9, and ARG = 9). The highest correlations ($P < 0.001$) between the two methods were recorded for DM ($r = 0.930$), CP ($r = 0.860$), CF ($r = 0.610$), NDF ($r = 0.690$), and gross energy ($r = 0.740$). Also, the correlation was significant for ash ($r = 0.580$; $P < 0.01$) and phosphorus ($r = 0.490$; $P < 0.05$). The most limiting AA in non-ruminants diets that showed significant correlation between wet chemistry and NIRS were Ile ($r = 0.580$), Leu ($r = 0.590$) with $P < 0.01$, and Lys ($r = 0.380$), Thr ($r = 0.440$), and Arg ($r = 0.460$) with $P < 0.05$. However, no correlations between wet chemistry and NIRS for EEh and protein quality indicators were found. The correlations found were lower than expected for many of the variables compared because the data on wet chemistry and the NIRS analyses were conducted in two different labs. It is concluded that the NIRS technology could be an alternative for the estimation of the chemical constituent of commercial SBM, including that of certain amino acids. However, more studies are needed to improve the accuracy of values obtained by NIRS for EEh and protein quality indicators.

Key Words: amino acids, near-infrared spectroscopy

technology, protein quality indicators, proximal chemical composition, soybean meal, wet chemistry

186 Effect of genetically modified DP-3Ø5423-1 (305423) soybean on immune response and fatty acid metabolism markers of broilers. Ali Calik*^{2, 1}, Nima Emami^{2, 4}, Mallory B. White², Rami A. Dalloul^{2, 3}; ¹*Animal Nutrition and Nutritional Diseases, Ankara University Faculty of Veterinary Medicine, Ankara, Turkey*, ²*Avian Immunobiology Laboratory, Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, Virginia, United States*, ³*Poultry Science, University of Georgia, Athens, Georgia, United States*, ⁴*Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States.*

Soybean plants containing the event DP-3Ø5423-1 were generated by introducing the gm-fad2-1 gene fragment and the gm-hra gene. The endogenous fatty acid desaturase-2 enzyme (FAD2-1) is highly expressed in the developing soybean seed especially during the period of oil deposition and is responsible for the conversion of oleic acid to linoleic acid. Transcription of the gm-fad2-1 gene fragment suppresses endogenous FAD2-1 resulting in higher levels of oleic acid and reduced levels of linoleic acid, linolenic acid, and to a lesser extent palmitic acid in the seed. The aim of this study was to evaluate the nutrient composition of high-oleic transgenic full-fat soybean meal (FFSBM; DP-3Ø5423-1) and near isoline non-transgenic control FFSBM, and also assess their feeding effects on mRNA abundance of immune response and fatty acid metabolism markers in broilers. Prior to trial initiation, proximate, fatty acid and amino acid analyses of these FFSBMs were determined and no differences were observed except for fatty acid composition. As expected, transgenic FFSBM contained a substantially higher proportion of oleic acid (C18:1; 76.37%) and lower proportion of linoleic and linolenic acids (18:2; 6.44%, C18:3; 2.03%) compared to the control FFSBM (C18:1; 18.17%, C18:2; 52.25%, C18:3; 10.58%). A total of 480 day-of-hatch chicks (Ross 308) were randomly allocated to 24 floor pens in a 2×2 factorial arrangement that included diet and gender (6 pens/group). Birds were fed isocaloric and isonitrogenous diets containing 20% of either DP-3Ø5423-1 or control FFSBM for 35 days. On d 35, two birds from each replicate were selected based on average pen BW. Spleen and liver tissue samples were excised to assess the mRNA abundance of immune response and fatty acid metabolism related genes, respectively. Data were subjected to a 2-way ANOVA using the GLM procedure of JMP (Pro13). There was no main effect of diet or gender on mRNA abundance of IL-1 β , IL-2, IL-6, IL-12b, IFN γ , TNF- α , and NF- κ B. Liver malic enzyme 1 (ME1) mRNA level was significantly lower in birds fed transgenic FFSBM diets. However, dietary treatment had no significant impact on ACC α , FAS, MTTP, SREBF, PPAR α , PPAR γ , and AMPK mRNA levels in the

liver. In conclusion, our results showed that genetically modified DP-3Ø5423-1 soybean is nutritionally equivalent to non-transgenic near-isoline counterpart with a comparable genetic background as evidenced by feed analyses except for fatty acid composition. Furthermore, transgenic FFSBM has no adverse effect on mRNA abundance of cytokines, transcription factors, and liver fatty acid metabolism markers.

Key Words: broiler, DP-3Ø5423-1, high-oleic soybean, mRNA abundance, transgenic soybean

187 Evaluation of increasing levels of enzyme-treated soy protein on turkey poult live performance. Kyle D. Brown*¹, Alfred Blanch², Meghan Schwartz³, Hannah Robinson³, Simone H. Rasmussen²; ¹*Hamlet Protein, Inc., Findlay, Ohio, United States*, ²*Hamlet Protein A/S, Horsens, Denmark*, ³*Applied Poultry Research LLC, Louisburg, North Carolina, United States*.

Soybean meal (SBM) is the preferred protein source in poultry dietary formulations due to its favorable amino acid profile. However, with increasing popularity of all vegetable diets, higher inclusion rates of SBM to satisfy the high protein requirements of turkey poult contributes concerning levels of anti-nutritional factors (ANF) that may have a detrimental effect on health and growth performance of these immature birds. One nutritional strategy to mitigate the negative effects associated with soy-ANFs is to include an alternative vegetable protein source and decrease the amount of SBM included in the diets. An experiment was conducted to evaluate live performance of increasing levels of enzyme-treated soy protein (ESP) in young turkey diets. 480 day-old female Nicholas Select poult were placed in 64 battery pens (7 or 8 birds/pen; 0.5 ft²/bird). A randomized complete block design was utilized, and replicate pens were assigned to one of four dietary treatments: 0%, 5%, 10%, or 20% ESP. Poult were fed a two-phase feeding program consisting of a starter (0-21 days) and grower (21-42 days). Dietary treatments in each phase were formulated to be isocaloric and contained similar amino acid ratios relative to digestible lysine based off breeder recommendations. All data were analyzed using one-way ANOVA and means deemed significantly different at $p \leq 0.05$ were separated using Student's *t*-test. Quadratic broken line and quadratic polynomial regressions were used to estimate optimal dose response of ESP. A significant effect of ESP inclusion was observed on FCR at 7 ($p=0.011$), 28 ($p=0.020$), 35 ($p=0.003$), and 42 ($p=0.030$) days of age. At each of these evaluation periods, a significant reduction in FCR was observed with 10% ESP compared to 0 and 5% with 20% ESP inclusion being intermediate. The quadratic polynomial regression was significant ($p=0.04$) for BW at day 42, with a breakpoint at 11.52% ESP, whereas the quadratic broken-line regression was not significant ($p=0.50$). For FCR in the grower period (21-42 days), the quadratic broken-line regression showed a near-significant trend ($p=0.09$) with a breakpoint of 5.9% ESP, whereas the quadratic polynomial regression did not ($p=0.35$). In

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conclusion, these data demonstrate the benefits of including 10% ESP in young poult diets through 42 days of age. The quadratic regressions estimate an optimal dose of ESP to be between 10-12% during the starter phase (0-21 days) and 5-12% during the grower phase (21-42 days).

Key Words: enzyme-treated soy protein, soybean meal, growth performance, turkey poult

188 Effects of dietary corn germ meal levels on growth performance, meat quality, serum biochemical indices, and intestinal morphology in Pekin ducks. Ying Y. Qi*, Keying Zhang, shiping Bai, Xuemei Ding, Jianping Wang, Yue Xuan, Zhuowei Su, huanwei peng, Qiufeng Zeng; *Sichuan Agricultural University, Cheng du, China*.

The experiment were carried out to determine the effects of dietary corn germ meal (CGM) levels on growth performance, carcass characteristic, meat quality, serum biochemical indexes, and intestinal morphology in Pekin ducks from 10 to 42 d of age. A total of 504 10-day-old Cherry Valley ducks were randomly allotted to 6 treatments with 6 replicate pens per treatment and 14 ducks per pen based on mean body weight. Six isonitrogenous and isocaloric experimental diets were formulated on a digestible amino acid basis to produce diets in which containing 0%, 3%, 6%, 9%, 12%, and 15% CGM, respectively. One-way analysis of variance (ANOVA) was performed for significance analysis among groups. All statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC) with $P < 0.05$ considered statistically significant, and post hoc comparisons of group means were carried out with Duncan's test when the ANOVA was significant. Results showed as follows: Compared with other groups, ducks fed 12% CGM significantly increased ($P < 0.05$) the feed to gain ratio and numerically increased the mortality. The percentage of breast meat weight and the content of crude protein in breast meat presented a linear decrease ($P < 0.05$) with the increase of dietary CGM levels. Serum biochemical indices (alanine aminotransferase, aspartate aminotransferase, glucose, high density lipoprotein cholesterol, total cholesterol, triglyceride, total protein and urea) showed no significant differences among all groups ($P > 0.05$). However, the serum content of low density lipoprotein cholesterol in 15% groups was significant lower ($P < 0.05$) than that in other groups. The jejunal crypt depth and the ileal ratio of villous height to crypt depth linearly increased ($P < 0.05$), but the ileal crypt depth and the jejunal ratio of villous height to crypt depth linearly decreased ($P < 0.05$) with the increase of dietary CGM levels. These results suggested that the optimal levels of CGM in diets for meat duck aged from 10-42d were 3% to 9%.

Key Words: corn germ meal, Pekin duck, growth performance, meat quality, optimal level

189 Influence of heat stress and the inclusion of sodium-butyrate in the diet, on growth performance, body weight uniformity, and intestinal morphology in

broilers from 0 to 39 d of age. V. Bernad¹, G. Fondevila*¹, B. Saldaña², J.L. Archs^{1, 2}, L. Aguirre¹, A.F. de Juan¹, Gonzalo Mateos¹; ¹UPM, Madrid, Madrid, Spain, ²Nutega S. L., Madrid, Spain.

The objective of this research was to study the effects of the inclusion of a protected form of sodium-butyrate in the diet on growth performance and morphology of the gastrointestinal tract in broilers from 0 to 39 d of age. The additive used was Butirex C4 (Novation 2002, Spain) a Na protected salt that contained by analyses 54% of butyrate and 21% of sodium. The feeding program consisted in three periods (0 to 20 d, 21 to 30 d and 31 to 39 d of age) and in each period the birds were fed one of two experimental diets. The two had the same composition and nutrient content with the only difference being the inclusion or not of 0.10% of sodium-butyrate. From 30 to 39 d of age, all birds were kept under a mild heat stress that consisted in increasing the average ambient temperature of the barn from 20 ± 2 to 30 ± 2 °C. The experimental design was completely randomized with 2 treatments and 18 replicate pens of 20 broilers each per treatment. BW gain, feed intake, and feed conversion ratio were analyzed by feeding period and cumulatively (0 to 39 d of age) using the MIXED procedure of SAS. The Tukey test was used to make pairwise comparisons to separate treatment means. At 29 and 39 d of age (beginning and end of the heat stress period) the birds were weighed individually and uniformity of the BW was estimated indirectly as the coefficient of variation (%) of the individual BW of the birds within each replicate. In addition, body temperature was measured in the cloaca in two birds per pen at 29, 32, and 39 d of age to measure the short-term (3 d) and long-term (10 d) response of the birds to heat stress. Also, villus height and the associated crypt depth of the jejunal mucosa were measured in two birds per replicate at 29 and 39 d of age. Sodium-butyrate increased BW gain from 0 to 21 d of age ($P < 0.05$) with most of the benefit observed for the first two weeks of life. At 39 d of age, one the birds were for 9 days under a mild heat stress situation, supplementing the diet with sodium-butyrate improved BW uniformity of the birds ($P < 0.05$) but did not affect the temperature of the cloaca or the morphology of the jejunal mucosa. In summary, the inclusion of Na-butyrate in the diet increased broiler performance for the first 2 wk of life. At 39 d of age, after 9 days of a mild heat stress, Na-butyrate improved BW uniformity of the birds although broiler growth was not affected.

Key Words: body weight uniformity, broiler, heat stress, intestinal morphology, sodium-butyrate

190 Multiple dietary supplementations of omega 3 fatty acids and calcidiol on growth performance, health status, and tissue enrichments of broiler chickens. Sahil Kalia*, Tao Sun, Andrew Magnuson, Ziqiao Sun, Xin Gen Lei; *Animal Science, Cornell University, Ithaca, New York, United States.*

The average US diet does not contain adequate long chain polyunsaturated fatty acids (PUFA), in particular omega 3

fatty acids DHA and EPA, and 25-hydroxy vitamin D₃ (calcidiol). Chicken meat is one of the most consumed animal-sourced proteins and an excellent candidate for enriching these nutrients. The present study was to determine effects of feeding microalgal DHA oil (Onavita, ADM, Decatur, IL), EPA-rich *Nannochloropsis* sp CO18 (MAGIC, Duke University, Beaufort, NC) and calcidiol (DSM, Parsippany, NJ) on growth performance, health status, and tissue enrichment of these nutrients in broiler chickens. Day-old Cornish male broiler chicks (total = 180) were divided in to 5 treatment groups (6 cages/treatment, 6 birds/cage). Birds were fed: Diet 1 = a corn-soybean meal basal diet (BD, control); Diet 2 = BD + DHA (1.5 g/kg for 0-3 wk and 3.0 g/kg for 4-6 wk); Diet 3 = Diet 2 + *Nannochloropsis* sp CO18 (0.3 g/kg for 0-3 wk and 0.6 g/kg for 4-6 wk); Diet 4 = Diet 2 + calcidiol (6,000 IU/kg for 0-3 wk and 12,000 IU/kg for 4-6 wk); and Diet 5 = Diet 3 + calcidiol (6,000 IU/kg for 0-3 wk and 12,000 IU/kg for 4-6 wk). Growth performance was recorded weekly. Blood, liver, breast, thigh, and adipose tissue were collected at wk 3 and 6 (2 chicks/cage) for biochemical analyses. Data were analyzed by one-way ANOVA. Birds fed Diet 3 had higher ($P < 0.05$) body weight gain and birds fed Diet 2 had higher ($P < 0.05$) gain:feed ratio (3.1-16%) at wk 6 than the other groups. Birds fed Diets 2-5 had higher ($P < 0.05$) DHA and n-3 PUFA concentrations (4-18-fold) and n3/n6 ratios (4-28-fold) in the liver, breast, and thigh than those in the control group at wk 6. Birds fed Diet 3 also had lower ($P < 0.05$, 8%-54%) concentrations of triglycerides, cholesterol, and non-esterified fatty acids in the plasma, liver, breast, and thigh than the control. Birds fed Diet 2 had lower ($P < 0.05$) concentrations of plasma (22%) and tissue phospholipids (31%-47%) than the control. The mRNA abundance of vitamin D metabolism genes (*CYP2R1*, *CYP24A1*, *VDR*) and lipid metabolism genes (*FASN*, *SREBP1*, *CPT1*) was upregulated in the liver by Diets 2-5 compared with the control at wk 3, whereas these genes were downregulated in breast, thigh and adipose tissue by Diets 2-5 compared with the control. Expression of *VDR*, *SREBP1* and *CPT1* in the liver, thigh, and adipose tissue was upregulated by Diets 2-5 compared with the control diet at wk 6. In conclusion, feeding chicks with supplemental omega 3 fatty acids and calcidiol improved growth performance, health status, and tissue accumulation of DHA of broiler chickens (Supported by DOE MAGIC grant DE-EE0007091, USDA grant 2019-69012-29905, and Cornell University Hatch grants NYC-127302).

Key Words: Broiler chicks, Calcidiol, DHA, Growth performance, Omega 3 fatty acids

191 No presentation materials submitted.

192 Effects of dietary DDGS levels on growth performance, serum biochemical indices, and intestinal morphology in Pekin ducks. Ying Y. Qi*, Keying Zhang, Shiping Bai, Jianping Wang, Xuemei Ding, Zhuowei Su, Yue Xuan, Qiufeng Zeng, Huanwei Peng; *Sichuan Agricultural University, Cheng du, China.*

This study was investigated to the effects of dietary corn distillers dried grains with solubles (DDGS) levels on growth performance, serum biochemical indices, and intestinal morphology in Pekin duck from 10 to 42 d of age. A total of 672 10-day-old Cherry Valley ducks were randomly allotted to 6 treatments with 7 replicate pens per treatment and 16 ducks per pen based on mean body weight. Six isonitrogenous and isocaloric experimental diets were formulated on a digestible amino acid basis to produce diets in which containing 0%, 5%, 10%, 15%, 20% and 25% DDGS, respectively. One-way analysis of variance (ANOVA) was performed for significance analysis among groups. All statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC) with $P < 0.05$ considered statistically significant, and post hoc comparisons of group means were carried out with Duncan's test when the ANOVA was significant. Results showed as follows: The mortality in 25% DDGS group (23.21%) was significantly higher ($P < 0.05$) than that in other groups, and the mortality of 10% DDGS group (1.79%) was the lowest among all treatments. Compared with 0%, 5%, 10% and 15% DDGS groups, the BW (d 42), ADG (d 10-42) and ADFI (d 10-42) in the 20% and 25% groups significantly reduced ($P < 0.05$), and feed to gain ratio significantly increased ($P < 0.05$). There was no significant difference ($P > 0.05$) on the growth performance of meat ducks in the 0%, 5%, 10% and 15% groups. No significant differences ($P > 0.05$) was observed on the contents of alanine aminotransferase, aspartate aminotransferase, glucose, high density lipoprotein cholesterol, low density lipoprotein cholesterol, total protein and urea in serum among all groups. However, the content of total cholesterol in 20% DDGS groups was significant higher ($P < 0.05$) than that in 0% and 5% DDGS groups, and the serum triglyceride contents in 0% and 5% DDGS groups was significant lower ($P < 0.05$) than in 15% and 20% DDGS groups. There was also no difference ($P > 0.05$) in the villous height of jejunum and ileum, the crypt depth of jejunum, and the ratio of villous height to crypt depth of jejunum and ileum among all treatments. Compared with other groups, the crypt depth of ileum significantly increased ($P < 0.05$) in 25% DDGS group. These results suggested that the optimal levels of DDGS in diets for meat duck aged from 10-42d were 5% to 10%.

Key Words: DDGS, Pekin duck, growth performance, serum biochemical indices, intestinal morphology

193 A comparison of statistical methods to estimate zinc bioavailability in chickens. Thi Thanh Hoai Nguyen¹, Robert A. Swick¹, Shubiao Wu¹, Gene M. Pesti^{*2, 1}, Lynne Billard³; ¹*School of Environmental and Rural Science, University of New England, Armidale, New South Wales, Australia*, ²*Poultry Science, University of Georgia, Greensboro, Georgia, United States*, ³*Statistics, University of Georgia, Athens, Georgia, United States*.

Ross 308 male broiler chicks (784) were fed 6 known levels of Zn hydroxychloride in a wheat/soybean meal-based basal starter (0 to 14 d) diet to form a standard curve (7 replicates

per level) with another 7 replicates fed a combination of 50 mg/kg Zn as ZnO and 50 mg/kg Zn as ZnSO₄ (Industry Reference Control) to compare responses to the standard curve. Various responses were measured when the chicks were 14 and 35 d old. Measured amounts of Zn (mg/kg) in starter were 31.0 to 135 the standard curve samples and 127.3 in the test sample. Of the various growth performance and tissue minerals measured, the most linear and highest R² (0.51) response was to tibia Zn concentration at 14 d. Using one-way ANOVA and Duncan's Multiple Range Test, no significant differences were found from birds fed the Reference Control Zn diet and ones fed 135 nor 73 mg Zn/kg from Zn Hydroxychloride. Using normal regression and inverse prediction, the Reference Control sample was estimated to contain 107.5 ± 15.7 mg Zn/kg diet (mean ± SEM). Using reverse regression and normal prediction, the Reference Control sample was estimated to contain 96.0 ± 8.1 mg Zn/kg diet. Using normal regression and Graybill's (1976) Abductive Method prediction, the Reference Control sample was estimated to contain 107.5 ± 6.1 mg Zn/kg diet. Since the abductive method is the only one to consider tibia Zn as a function of dietary Zn, and includes variation in the standard curve in its calculations, it is the most appropriate one to use. Relative bioavailability of the Industry Reference Control sample was 107.5 / 127.3 = 84.1% compared to the Zn hydroxychloride standard. This indicates higher bioavailability of (100/84.1 =) 119% for Zn hydroxychloride as compared to the commonly used combination of 1:1 ZnO and ZnSO₄.

Key Words: Zinc, Bioavailability, Statistics, Design, Calibration

194 An assessment of the variation in limestone grit originating from North and South America and implications for laying hen nutrition. Anneleen Swanepoel^{*1}, Gareth Wilks^{2, 5}, Janet Remus⁶, Mauricio S. Cunha⁶, Peter W. Plumstead³, Rosalina Angel⁴; ¹*Poultry, Chemuniqué (Pty) Ltd, Lanseria, Gauteng, South Africa*, ²*Poultry, Chemuniqué, Johannesburg, Gauteng, South Africa*, ³*Chemuniqué PTY LTD, Lanseria, South Africa*, ⁴*Animal and Avian Sciences, University Of Maryland, College Park, Maryland, United States*, ⁵*Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria, South Africa*, ⁶*Danisco Animal Nutrition (IFF), Cedar Rapids, Iowa, United States*.

Limestone (LS) is the primary source of Ca in laying hen diets, contributing as much as 94% of the Ca consumed by a hen. A large portion of the LS in commercial diets is supplied as large particle grit that is expected to be slowly soluble to provide Ca in the intestine at the time of shell formation. Since previous research has shown LS particle size and solubility to be negatively correlated with eggshell and bone quality in laying hens, it was of interest to understand the variation in grit LS used in commercial feed mills. Grit samples (109) with a geometric mean diameter (GMD) >1000 µm were collected from South America (SA) and North America (NA). Samples were subjected to

moisture analysis and nine minerals (Ca, Cu, Fe, Mg, Mn, P, K, Na, Zn) following the AOAC (2002), and AOAC (2016), adapted for ICP-OES. Particle size was determined using a shaker and a set of 14 sieves ranging from 3,35mm to 0,25mm plus a base pan. A 100g sample of each LS was subjected to 6 minutes of shaking and the GMD of LS particle by mass (dgw) was determined by using the equations as described by Wilcox et al. (1962). The solubility of each LS was determined in duplicate by making use of the dynamic solubility assay as described by Kim *et al.* (2019) at 30, 90, and 150 minutes (min). All data were analyzed using JMP® statistical package, Version 15.0. Analyzed Ca in SA LS was $37.1 \pm 2.0\%$ and $37.9 \pm 1.4\%$ for NA samples. The range in GMD particle size was 1001.3 to 3590.2 and 1095.84 to 3139.2µm for SA and NA samples, respectively. Solubility at 30-min ranged from 23.7 to 94.0% for SA samples and 16.6 to 86.3% for NA samples. The weak negative correlation between GMD particle size and 30-min solubility ($r=0.49$) indicated that particle size could only explain approximately 50% of the variation in solubility. For example, one LS sample from NA with a GMD of 2017 µm had a 30 min solubility of 71.3%; while a second sample with GMD of 2106 µm had a 30 min solubility of 45.0%. The poor correlation between GMD particle size and solubility, as well as large observed differences in the 30 min solubility of LS with a similar particle size emphasizes the shortcoming of only using LS GMD particle size as the sole criteria to select LS grit used in commercial laying hen diets.

Key Words: Limestone, Laying hen, Grit, Particle size, Shell quality

195 No presentation materials submitted.

196 Effects of dietary metabolizable energy and standardized ileal digestible lysine content of the diet on performance and egg quality of brown-egg laying hens from 18 to 41 weeks of age. R. Scappaticcio², H. Herrera², G. Fondevila¹, A.F. de Juan¹, L. Cámara¹, Gonzalo Mateos^{*1}; ¹UPM, Madrid, Madrid, Spain, ²Camar Agroalimentaria S.L., Toledo, Spain.

The influence of energy (AMEn, kcal/kg) and standardized ileal digestible lysine (SIDLys) content of the diet on egg production and egg quality traits 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 two energy concentrations (2,750 and 2,700 kcal/kg) and five AMEn to SIDLys ratios (3.48, 3.62, 3.77, 3.93, and 4.10). Each treatment was replicated 10 times and the experimental unit was the cage with 10 hens for all measurements. To ensure that the SIDLys 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 SIDLys content. Birds had free access to feed and water throughout the trial. Production data (egg rate, feed intake, egg weight, egg mass, FCR, BW gain, and mortality)

were collected and analyzed by period (6 periods of 4 wk each) 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 in the last 2 days of each of the 6 experimental periods. Data were analyzed as a completely randomized design using the MIXED procedure of SAS, with energy concentration, AMEn to SIDLys ratio, and the interaction as main effects. In addition, the effects of the level of SIDLys on the variables studied were partitioned into its lineal (L) and quadratic (Q) components. All differences were considered significant at $P < 0.05$. Nointeractions between AMEn and SIDLys level of the diet were found for any of the variables studied and therefore, only main effects are presented. An increase in the AMEn content of the diet from 2,700 to 2,750 kcal/kg increased egg weight ($P < 0.05$) but did not affect the number of eggs produced or feed conversion ratio. A decrease in the AMEn to SIDLys ratio, increased linearly egg weight ($P < 0.05$) and egg mass production ($P < 0.05$) and tended to improve feed conversion ratio ($P = 0.100$). Feed intake and BW gain, however, were not affected. Neither energy nor AMEn to SIDLys ratio affected any of the egg quality traits studied. However, shell resistance to breakage increased linearly ($P < 0.05$) as the AMEn to SIDLys ratio of the diet decreased. In conclusion, an increase in the energy content of the diet from 2,700 to 2,750 kcal AMEn/kg did not affect egg production but increased egg weight and egg mass production. The data indicate that brown laying hens require no more than 769 mg SIDLys/d to optimize egg production. However, when the objective is to maximize egg weight, at least 806 mg SIDLys/d are needed.

Key Words: egg production, energy, egg weight, laying hens, standardized ileal digestible lysine

197 Egg quality and serum biochemical profile of laying breeder hens fed diets supplemented with probiotic and organic chromium. Otoniel F. Souza¹, Catarina Stefanello¹, Willian Gräf¹, Beatriz N. Rodrigues¹, Geovana Müller¹, Elisa François², Kelen Zavarize^{*2}; ¹Animal Sciences, Federal University of Santa Maria, Santa Maria, Brazil, ²Kemin Industries Inc., Valinhos, SP, Brazil.

The objective of this study was to evaluate the effects of probiotic (*Bacillus subtilis* PB6), organic chromium (Cr propionate) or their combination on egg and eggshell quality as well as serum biochemical profile of laying breeder hens. A total of 32 Rhodes Island Red and 32 White Plymouth Rock breeder hens at 55 weeks of age were allocated in individual wire cages (0.33 m x 0.46 m x 0.40 m) using a completely randomized block design with 16 replicates. Hens were fed 4 experimental feeds in 4 periods of 28 days each, from 55 to 70 wks. Experimental feeds were a Control diet (formulated with corn, soybean meal and wheat bran without additives), Control + Probiotic (supplemented with 500 g/ton of *Bacillus subtilis* PB6; CLOSTAT); Control + Cr (50 g/ton of organic Cr; KemTRACE), and Control + Probiotic + Cr (with both

additives). In the last 4 days of each experimental period, all eggs were collected to evaluate: egg weight, specific egg weight, percentage of albumen, yolk and shell, yolk index and Haugh unit as well as shell thickness and shell strength. Blood was collected from 8 birds per treatment every period to determine cortisol, albumin, glucose, total cholesterol, and triglycerides serum concentration. Data were subjected to one-way analysis of variance using the MIXED procedure of SAS Institute and significance was accepted at $P < 0.05$. Means were compared by Tukey test. No effect was observed between the hens' strain, and only dietary treatments were considered as independent variable. Treatments did not affect egg weight, Haugh unit, yolk index, total solids and percentage of albumen and yolk ($P > 0.05$). From 63 to 66 wk, 67 to 70 wk and 55 to 70 wk, hens fed Control + Probiotic, or Control + Cr or Control + Probiotic + Cr had higher ($P < 0.05$) shell percentage than hens fed the Control. In all evaluated periods, shell strength increased when hens were fed Control + Cr or Control + Probiotic + Cr compared to the Control not supplemented, also having higher specific weight and shell thickness. There were no differences ($P > 0.05$) among periods and dietary treatments on cortisol, albumin and triglycerides; however, in the overall period, cholesterol and glucose serum concentration decreased ($P < 0.05$) when hens were fed Control + Probiotic + Cr compared to the Control. In conclusion, laying breeder hens fed diets supplemented with probiotic, organic chromium or their combination produced eggs with increased eggshell quality, presenting increased shell strength, shell thickness and specific weight, without modifying the main serum biochemical parameters.

Key Words: *Bacillus subtilis*, breeder hen, chromium propionate, cortisol, eggshell quality

198 Apparent metabolizable energy and performance of broilers and Japanese quail fed selected modern grain sorghum varieties. Alissa Moritz*^{GS 1}, Shanice Krombeen¹, Michael Blair², Bob Buresh³, Richard Kaminski⁴, William Bridges⁵, Mireille Arguelles-Ramos¹, Tiffany Wilmoth¹; ¹*Animal and Veterinary Sciences, Clemson University, Clemson, South Carolina, United States*, ²*United Animal Health, Sheridan, Indiana, United States*, ³*Novus International, St. Charles, Missouri, United States*, ⁴*Department of Forestry and Environmental Conservation, Clemson University, Clemson, South Carolina, United States*, ⁵*Department of Mathematical and Statistical Sciences, Clemson University, Clemson, South Carolina, United States*.

Intensive pressures on corn production for poultry and gamebird diets are a major concern due to the increased competition among human, animal and industrial users. To reduce these pressures, alternative feedstuffs are of great interest. An alternative feedstuff that is comparable to corn is grain sorghum (milo). Modern varieties of U.S. grain sorghum are nearly 99% tannin-free; however, limited data are available to support using modern varieties as an alternative feedstuff to effectively replace corn in poultry

and gamebird feeds. The objective of this study was to determine the apparent metabolizable energy (AME_n) content of red/bronze, white/tan and U.S. No. 2 yellow varieties of grain sorghum for feeding commercial broilers and Japanese quail. AME_n response was evaluated during two phases of grow-out for commercial broilers ($n = 224$; 22-24 day of age; 43-45 day of age) and Japanese quail ($n = 314$; 18-20 day of age; 36-38 day of age). Both species of birds were phase-fed 1 of 4 corn basal diets with 20% of the calories for the gross energy of corn replaced by the equivalent calories of the gross energy of milo for treatment diets or dextrose for the control diet. During each phase, feed intake and excreta was measured for nitrogen and gross energy content. AME_n was determined using the difference method. Weekly measures of mean body weight and feed consumption were used to calculate body-weight (BW), feed intake (FI), and feed conversion ratio (FCR). Analyses were based on a 2 x 4 factorial treatment design with age (grower and finisher-phases) and grain types (corn-dextrose, red/bronze, white/tan, and U.S. No. 2 yellow) defining the treatments, and a randomized complete block experiment design with cage and trials defining the block. For broilers, the AME_n values were significantly different ($P < 0.05$) among treatments for both phases, while FI and FCR were significantly different ($P < 0.05$) in the grower phase only. For the Japanese quail, AME_n values were different ($P < 0.05$) among treatments in the grower phase. Overall, the mean AME_n values of milo varieties were 3,420 kcal/kg (white/tan), 3,181 kcal/kg (U.S. No. 2 yellow), and 3,363 kcal/kg (red/bronze) for broilers. In Japanese quail, mean AME_n values of milo varieties were 3,266 kcal/kg (white/tan), 2,988 kcal/kg (U.S. No. 2 yellow), and 3,449 kcal/kg (red/bronze). Results suggest the potential for using these respective milo varieties as a replacement for corn in diet formulation without adverse effects on growth performance factors.

Key Words: apparent metabolizable energy, broiler, grain sorghum, gamebird, Japanese quail

199 Effect of select modern grain sorghum varieties on the growth performance and carcass traits of broiler chickens. Alissa Moritz*^{GS 1}, Michael Blair³, Bob Buresh⁴, William Bridges², Mireille Arguelles-Ramos¹; ¹*Animal and Veterinary Sciences, Clemson University, Clemson, South Carolina, United States*, ²*Department of Mathematical and Statistical Sciences, Clemson University, Clemson, South Carolina, United States*, ³*United Animal Health, Sheridan, Indiana, United States*, ⁴*Novus International, St. Charles, Missouri, United States*.

Corn constitutes 60-70% of poultry diets, and its direct competition with human and industrial users makes it favorable to seek more sustainable, alternative feed ingredients. U.S. grain sorghum, which is 99% tannin-free, may be a viable alternative to corn due to its similar nutritional profile. Assessment of growth performance parameters may elucidate perceived issues and offer producers an adequate recommendation for use of grain

sorghum as an alternative feed ingredient in poultry production. The objective of this study was to evaluate the effect of modern varieties of grain sorghum (red/bronze, white/tan, and U.S. No. 2 yellow) and validate their previously determined nutrient profiles on the growth performance and carcass traits of Cobb 500 x Hubbard male broilers (n = 640) at full-substitution of corn. Birds (20 birds/pen and 8 pens/treatment) were fed 1 of 4 treatments (corn, red/bronze sorghum, white/tan sorghum or U.S. No.2 yellow sorghum) of crumble/pellet feed according to a three-phase feeding program: starter (1 to 14 d-of-age), grower (15 to 28 d-of-age) and finisher (29 to 41 d-of-age). Group body weight (BW) and feed intake (FI) were recorded weekly and mortality weighed and recorded daily to calculate average BWG,FI and adjusted feed conversion ratio (AdjFCR). On day 41, 2 birds from each pen/treatment was randomly selected for slaughter and further processing to obtain carcass yield (CY) and breast yield (BY). The model effects were assessed with one-way ANOVA based on the treatment and experiment design, and Fisher's Least Significant Difference procedure was used to determine specific differences among the grain type means. At 41 d-of-age, birds fed the corn treatment were heavier ($P < 0.05$) than those fed white/tan sorghum, while red/bronze and U.S. No. 2 yellow sorghum treatments were intermediate. FI was higher for standard corn treatment, when compared to red/bronze, white/tan and U.S. No. 2 yellow sorghum treatments ($P < 0.05$). No significant differences were observed for AdjFCR, CY and BY among treatments. This study indicated that modern grain sorghum varieties may be an adequate alternative to corn in poultry diets without negatively impacting performance and carcass parameters.

Key Words: alternative feedstuff, broilers, grain sorghum, growth, performance

200 Effect of beak trimming at hatch and oat hulls inclusion in the diet on growth performance, pecking behavior, and digestive tract traits of brown-egg pullets from hatch to 15 weeks of age. J. Ben Mabrouk*^{GS}, Gonzalo Mateos, A.F. de Juan, B. Carreño, V. Bernad, L. Cámara; *UPM, Madrid, Madrid, Spain.*

The influence of oat hulls (OH) inclusion in the diet on growth performance, pecking behavior, and gastrointestinal tract traits were studied in brown pullets with intact or infrared-trimmed beak at hatch from 0 to 15 wk of age. The experiment was conducted as a completely randomized design with 4 treatments arranged as a 2×2 factorial with beak treatment (intact beaks vs. trimmed at hatch) and OH inclusion (0 vs. 3%) as main effects. Each treatment was replicated 20 times and the experimental unit was an enriched cage with 10 birds. Pullet performance, [feed intake, BW gain, and feed conversion ratio (FCR)] were determined by period (0 to 6, 6 to 10, and 10 to 15 wk of age) and cumulatively. Also, energy intake (kcal AMEn/d) and energy conversion ratio (kcal AMEn/g BW gain) were estimated at same ages. Feed preference behaviour was determined at 6 wk of age by measuring the difference in

geometric mean diameter and the geometric standard deviation of the feeds at 08.00 am (lights on) and 24 hours after. The exploratory pecking behavior was evaluated at 8, 10, 12, and 14 wk of age as a) time elapsed from the introduction of a ballpoint pen in the lateral panel of the cage until a first pullet pecked it and b) number of pullets within each cage that pecked the ballpoint pen in less than 5 seconds. Also, at 10 and 15 wk of age, two birds per replicate were selected at random, sacrificed, and weighed individually. The proventriculus, gizzard, the small intestine, and the large intestine were excised for further determinations. Data were analyzed for beak trimming and OH inclusion as main effects, and their interactions using the MIXED procedure of SAS. No interactions between main effects were detected for any of the traits studied and therefore, only main effects are presented. From 0 to 15 wk of age, beak trimming improved FCR ($P < 0.01$) but did not affect any other trait. Beak trimming increased ($P < 0.05$) mortality from 0 to 6 wk but not thereafter. Oat hulls inclusion tended to improve energy efficiency ($P = 0.084$) and BW uniformity ($P = 0.090$) but did not affect BW gain. Preference for coarse particles was reduced in trimmed birds. Beak trimming and OH feeding reduced pecking activities at all ages ($P < 0.05$). Oat hulls increased the weight of the gizzard and the small and large intestine at all ages ($P < 0.05$). In summary, beak trimming at hatch improved FCR and reduced the exploratory pecking behavior of the birds. Oat hulls increased gizzard weight at all ages and improved energy efficiency but did not affect BW gain. In addition, OH reduced the exploratory pecking behavior of the birds.

Key Words: beak trimming, brown pullet, gastrointestinal tract trait, oat hull, pecking behavior

201 An assessment of the variation in limestone quality originating from North and South America and implications for broiler nutrition. Gareth Wilks*^{GS 1, 4}, Anneleen Swanepoel¹, Janet Remus³, Mauricio S. Cunha³, Peter W. Plumstead¹, Rosalina Angel²; ¹*Chemuniqué PTY LTD, Lanseria, South Africa*, ²*Animal and Avian Sciences, University of Maryland, College Park, Maryland, United States*, ³*Danisco Animal Nutrition (IFF), Cedar Rapids, Iowa, United States*, ⁴*Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria, South Africa.*

In vegetable-based broiler diets, limestone (LS) can contribute up to 70% of the total dietary Ca supplied to broilers. Recent research has further shown that LS particle size and solubility alters both Ca digestibility and phytase efficacy in broilers. This makes it of interest to quantify variation in LS included in commercial broiler diets. A total of 200 fine LS samples with a geometric mean diameter (GMD) of $< 1000 \mu\text{m}$ were collected from feed mills across North America (NA) and South America (SA). Of these 200 samples, 64 of the samples originated from 6 countries in SA with the remainder being sourced from the USA and Canada. Samples were analyzed for moisture and nine minerals (Ca, Cu, Fe, Mg, Mn, P, K, Na, Zn) following the

AOAC (2002), and AOAC (2016), adapted for ICP-OES. Particle size was determined using a shaker and a set of 14 sieves ranging from 3,35mm to 0,25mm plus a base pan. A 100g sample of each limestone was subjected to 6 minutes of shaking and the GMD of limestone particle by mass (dgw) was determined using the equations as described by Wilcox et al. (1962). The solubility of each LS was determined in duplicate by making use of the dynamic solubility assay as described by Kim et al. (2019) at 5, 15, and 30 minutes (min). All data were analysed using JMP statistical package, version 15.0. Analyzed Ca for NA was $37.9 \pm 1.7\%$ and $37.2 \pm 2.3\%$ for SA with a range of 31.6-40.0% and 30.4-39.9%, respectively. The respective GMD particle size of LS from NA and SA was 378,55 and 300,51 microns, respectively. Only 15% of the LS sampled were less than 150 micron indicating the coarser nature of fine LS used. Solubility of LS at 5, 15, and 30 min was $60.0 \pm 17.2\%$, $80.9 \pm 11.7\%$ and $90.8 \pm 7.3\%$ respectively. There was a moderate negative correlation ($r=0.59$) between GMD particle size and solubility at 5 min for all 200 samples, indicating that as particle size increased solubility decreased. However, there were many exceptions to the rule. For example, two samples one from Costa Rica and another from Canada had very different GMD particle size (457.7 vs 283.8) but both solubilized 59.7 % at 5 min. This shows that in addition to particle size, differences in the limestone rock geology can influence solubility rate. The large variation in GMD particle size and dynamic solubility of LS used in commercial broiler diets can be expected to contribute to differences in Ca and P utilization by the bird, and should be considered in practical diet formulation.

Key Words: Calcium, Limestone quality, Particle size, Solubility, Broiler

202 Effects of encapsulated cinnamaldehyde on intestinal digestive and absorptive functions, meat quality and gut microbiota in broiler chickens. Chongwu Yang*^{GS 1}, Moussa Diarra², Janghan Choi¹, Argenis Rodas-Gonzalez¹, Dion Lepp², Shangxi Liu¹, Peng Lu¹, Marion Mogire¹, Qi Wang², Joshua Gong², Chengbo Yang¹; ¹*Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada*, ²*Agriculture and Agri-Food Canada, Guelph, Ontario, Canada*.

Essential oils are potential antimicrobial alternatives and their applications in animal feeds are limited due to their fast absorption in the upper gastrointestinal tract. The objective of this study was to investigate the effects of encapsulated cinnamaldehyde (CIN) at 50 mg/kg or 100 mg/kg on organ weights, meat quality, intestinal morphology, jejunal gene expression, nutrient digestibility, and ileal and cecal microbiota. A total of 320 male day-old broiler Cobb-500 chicks were randomly allocated to 4 treatments with 8 pens per treatment (10 birds per pen): 1) basal diet (negative control, NC); 2) basal diet supplemented with 30 mg/kg avilamycin premix (positive control, PC); 3) basal diet with 50 mg/kg encapsulated CIN (EOL); 4) basal diet with 100 mg/kg encapsulated CIN (EOH). The data was analyzed by

one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test (SAS 9.4). Despite birds fed EOH tended to increase ($P = 0.05$) meat pH at 24 h, all pH values were normal. Similar to the PC group, meat from birds fed EOL and EOH showed a reduced ($P < 0.05$) Warner-Bratzler force shear (WBFS) compared to the NC group. The highest villus to crypt ratio (VH/CD; $P < 0.05$) was observed in broiler fed either EOL or EOH, with an average being 14.67% and 15.13% in the duodenum and 15.13% and 13.58% in the jejunum, respectively. For jejunal gene expressions, only six out of the 11 studied genes showed statistical differences among the dietary treatments. Gene expressions of cationic amino acid transporter 1 (CAT-1) and neutral amino acid transporter 1 (B⁰AT-1) were upregulated in EOH-fed birds compared to PC and NC-fed birds ($P < 0.05$), respectively; while expression of proliferating cell nuclear antigen (PCNA) was downregulated in EOL-fed birds when compared to NC birds ($P < 0.05$). Nonetheless, the expressions of cadherin 1 (CDH-1), zonula occludens 1 (ZO-1), and maltase-glucoamylase (MG) were all upregulated ($P < 0.05$) in EOH-fed birds compared to PC-fed birds. The apparent ileal digestibility (AID) of dry matter, crude protein, crude fat and of all 18 tested amino acids increased in EOL-fed birds ($P < 0.01$). Additionally, relative abundances (%) of ileal Proteobacteria decreased, while ileal and cecal *Lactobacillus* increased in EOH-fed birds ($P < 0.05$). In conclusion, dietary encapsulated CIN improved meat quality and gut health by reducing meat WBFS, increasing VH/CD in intestines, jejunal gene expressions, AID of nutrients, and beneficially ileal and cecal microbiota composition.

Key Words: Antimicrobial alternatives, Broiler chickens, Encapsulated essential oils, Gut health, Meat quality

203 Supplemental dietary omega 3 fatty acids and calcidiol improved intestinal integrity and function of broiler chickens. Tao Sun^{GS *}, Sahil Kalia, Andrew Magnuson, Xin Gen Lei; *Animal Science, Cornell University, Ithaca, New York, United States*.

Previous research demonstrated the beneficial effects of supplementing omega 3 fatty acids (DHA & EPA), vitamin D, or microalgae alone on intestinal health of broiler chickens. The present study was to determine collective effects of supplemental microalgal DHA oil (Onavita, ADM, Decatur, IL), EPA-rich *Nannochloropsis* sp CO18 (Duke University, Beaufort, NC) and/or calcidiol (DSM, Parsippany, NJ) on intestinal integrity of broilers. Day-old Cornish male broiler chicks (total = 180) were grouped into 5 treatments (6 cages/treatment, 6 birds/cage) and fed: Diet 1: a corn-soybean meal basal diet (BD, control); Diet 2: BD + DHA oil (1.5 g/kg); Diet 3: Diet 2 + *Nannochloropsis* sp CO18 (0.3 g/kg); Diet 4: Diet 2 + calcidiol (6,000 IU/kg); or Diet 5: Diet 3 + calcidiol (6,000 IU/kg) for 3 wk. Blood, duodenum, jejunum and ileum tissues were collected (2 chicks/cage) at the end after orally gavaged with 2 sequential daily doses of dextran sulfate sodium (0.5 g per

chick), followed by 1 dose of fluorescein isothiocyanate dextran (FITC-d, 8.32 mg/kg body weight) as the indicator of gut permeability. Data were analyzed by one-way ANOVA. Diets 2-5 decreased ($P < 0.05$) serum FITC-d concentrations by 24% to 33% compared with Diet 1. Diet 2 increased ($P < 0.05$, 29%) jejunal crypt depth and Diet 3 increased ($P < 0.05$, 12%) jejunal villi height than the control. However, Diet 2 decreased ($P < 0.05$) ileal crypt depth and villi height by 13% and 24%, respectively, than the control. Gene transcripts of tight junction proteins including claudin-1, claudin-5, zonula occludens-1 (ZO-1), ZO-2, junctional adhesion molecule 2 (JAM-2), and JAM-3 in the small intestines were affected by Diets 2, 4 and/or 5 compared with the control. Diets 2, 4, and 5 decreased mRNA levels of jejunal claudin-1 gene (37%-40%, $P < 0.05$), and Diet 5 decreased those of JAM2 and JAM3 ($P < 0.05$, 35% - 47%). Diet 5 also caused similar decreases ($P < 0.05$, 40%) in mRNA levels of duodenal claudin-1 compared with the control. In contrast, Diets 2, 3, and 4 up-regulated ($P < 0.05$, 61% to 1.9-fold) mRNA levels of ZO-1 and ZO-2 in the ileum than the control. Diets 4 and 5 elevated ($P < 0.05$) duodenal claudin-1 protein by 29 to 47% over the control. Diets 2, 3 and 4 up-regulated ($P < 0.05$) duodenal ZO-1 protein by 54% to 82% over the control. In conclusion, the double and triple supplementations of DHA, EPA, and calcidiol into diets for broiler growers improved intestinal integrity and enhanced its resistance to exogenous insults via up-regulating functional expression of tight junction proteins in the small intestines (Supported by DOE MAGIC grant DE-EE0007091, USDA grant 2019-69012-29905, and Cornell University Hatch grant NYC-127302).

Key Words: broiler chicks, gut integrity, microalgae, omega 3 fatty acid, tight junction protein

204 Complete replacement of soybean meal with black soldier fly larvae meal in feeding program for broiler chickens depressed growth performance from placement through to 49 days of age. Hannah M. Facey*^{GS1}, Munene Kithama², Mohsen Mohammadigheisar¹, Lee-Anne Huber¹, Anna Kate Shoveller¹, Elijah Kiarie³; ¹Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, ²Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, ³Animal Biosciences, University of Guelph, Guelph, Ontario, Canada.

Black soldier fly larvae meal (BSFLM) is an appealing ingredient possessing good quality protein and additional functional attributes. However, there is limited data on the inclusion of BSFLM in practical broiler rations. Therefore, we examined the influence of partial to complete replacement of soybean meal (SBM) with BSFLM in a broiler feeding program. A total of 1,152 d-old male Ross × Ross 708 chicks were allocated to 48 floor pens and assigned to one of six diets in a randomized complete block (room) design (n=8). The diets were a basal (0% BSFLM) corn-SBM diet (A) with no prebiotic, probiotic, anticoccidial or antimicrobial growth promoting substances, four BSFLM diets formulated to replace SBM in A diet by

12.5% (B), 25% (C), 50% (D), and 100% (E), and diet (F), diet A plus coccidiostat (Monteban) and antibiotic. To provide similar energy among treatments, soy oil was used for diets A and F and black soldier fly oil for the other diets. The mash diets had similar nutrient specifications and were prepared for three phases: starter (d 0-10), grower (d 11-24), and finisher (d 25-49). Body weight (BW), feed intake (FI), and mortality (dead and compromised birds) were recorded on a per pen basis for calculation of BW gain (BWG) and mortality-corrected FCR. The data were analyzed using the Glimmix procedures of SAS with fixed effect of diet and random effect of block. Tukey's test was used to separate LSmeans and linear and quadratic contrasts were applied for BSFLM responses. In the starter phase, birds fed diets B, C and F had higher ($P < 0.01$) BWG than birds fed diets A, D, and E, however, birds fed diet E had lower ($P < 0.01$) BWG than birds fed diets A or D. Birds fed diets D and E had lower ($P < 0.05$) BWG than birds fed other diets in the grower and finisher phases. Birds fed diet F had higher final BW (3,257 g) than birds fed other diets. The final BW of birds fed diets A (2,997 g), B (3,068 g), and C (3,068 g) were similar but higher ($P < 0.01$) than for birds fed diet D (2,749 g) and E (2,450 g). Overall (d 0-49), replacement of SBM with BSFLM linearly decreased ($P < 0.01$) BWG and FI and increased ($P < 0.01$) FCR and mortality. The BWG and FI of birds fed 50 and 100% BSFLM diets was 96 and 92%, 90 and 82% of birds fed 0% BSFLM (diet A), respectively. The data indicated that birds fed lower levels of BSFLM (12.5% and 25%) outperformed the basal diet fed birds and could provide some growth promoting effects commensurate to coccidiostats and antibiotics. However, replacing SBM with greater amounts ($\geq 50\%$) of BSFLM reduced growth linked to depressed feed intake. Further research is warranted to explore physiological basis of depressed growth when birds are fed higher amount of BSFLM.

Key Words: Broiler chickens, Black Soldier Fly Larvae Meal, Growth Performance

205 Energy values of *Tenebrio molitor* larvae meal for broiler chickens determined using the regression method. Yuri Katagiri Dalmoro*^{GS}, Carine Adams, Lidiele Bairros, Guilherme L. Godoy, Beatriz N. Rodrigues, Marina Botega Lang, Catarina Stefanello; *Department of Animal Science, Federal University of Santa Maria, Santa Maria, RS, Brazil.*

Insect meal represents a new field of study as an ingredient for animal nutrition. This ingredient is rich in protein and energy and can be produced under a sustainable view. In the current study, the ileal digestibility energy (IDE), apparent metabolizable energy (ME), and nitrogen-correction ME (ME_n) of *Tenebrio molitor* larvae meal were determined using the regression method. A total of 189 slow feathering Cobb 500 male broilers were fed 3 experimental diets with 9 replicate cages of 7 birds each, in a completely randomized design. Broilers were fed a corn-soybean meal reference diet (RD) and 2 test diets (TD) from d 14 to 21

post-hatch. The TD consisted of *Tenebrio molitor* larvae meal that partly replaced the energy sources in the reference diet at 100 or 200 g/kg, such that equal rations were maintained for all energy-containing ingredients across all experimental diets. Excreta samples were collected twice daily from d 19 to 21, and ileal digesta were collected on d 21. Data were analyzed using the GLM procedure of SAS. Regressions of the test ingredient-associated IDE, ME, or ME_n intake in kilocalories against kilograms of test ingredient intake for cage of birds was conducted using linear regression following SAS statements. Apparent ileal digestibility of dry matter (DM), nitrogen (N), and amino acids as well as the total tract metabolizability coefficients of DM, N, and energy were calculated. The addition of *Tenebrio molitor* larvae meal to the RD linearly increased ($P < 0.05$) ileal and total tract of DM and N digestibilities as well as IDE, ME, and ME_n ($P < 0.05$). The inclusion of increasing levels of *Tenebrio molitor* larvae meal to the RD did not result in quadratic effects for all evaluated performance responses ($P > 0.05$). The IDE, ME, ME_n values of *Tenebrio molitor* larvae meal for broilers were 5,140, 5,238, and 4,925 kcal/kg of DM, respectively. In conclusion, the current study showed that broilers can utilize a considerable amount of energy and amino acids from tilapia byproduct meal; however, as with many new products, the nutrient can vary, and more data is needed to create databases to formulate diets for broilers chickens.

Key Words: broiler, digestibility, insect meal, nutrition, *Tenebrio molitor*

206 Spirulina (Arthrospira) platensis ingredient characterization and amino acid digestibility in male Cobb 500 broilers. Garrett Mullenix*^{GS 1}, Craig W. Maynard³, Samuel J. Rochell¹, Walter G. Bottje¹, Roy Brister², Michael T. Kidd¹; ¹*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*, ²*Tyson Foods Inc., Springdale, Arkansas, United States*, ³*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*.

Spirulina Platensis, a blue green-micro algae, is growing in interest as feed ingredient for poultry as it typically has a higher protein level than soybean meal while also being dense in vitamins, minerals, and pigments. Three experiments were conducted to determine apparent metabolizable energy corrected by nitrogen (AMEn), apparent ileal amino acid digestibility (AIAD), standard apparent ileal amino acid digestibility (SIAD), and Spirulina efficacy. The AMEn and SIAD values determined were used to conduct the subsequent Spirulina efficacy experiment via a titration. Both digestibility experiments were ran on wire floored metabolic cages (27" x 24" x 15") with Cobb 500 by-product males. Three hundred and eighty-four chicks were reared in group cages (8/chicks/cage) and fed a standard starter ad libitum until 14d. Each cage was then randomly allocated to a digestibility trial for AMEn determination through the total excreta collection method or to amino acid analysis through

the indigestible biomarker titanium oxide method. For the titration experiment 720 male Cobb 500 chicks (12/birds/pen) were reared in (3' x 4' x 2') floor pens and fed a standard starter ad libitum until 14d. The experimental diets were formulated to be iso-caloric and iso-nitrogenous while containing Spirulina at 0-5% (0,1, 2, 3, 4 and 5%) to 49 d of age. Performance parameters, carcass yields, meat quality, meat colorimetric and footpad scores were calculated (n=5). Data analysis was performed by using JMP Pro15 statistical analysis software (SAS Institute, 2018) for ANOVA with $P < 0.05$ indicating significant differences. Linear regression was further used to determine the AMEn and analyze breast meat color. Two batches of Spirulina were sourced from the same supplier for these experiments and average proximate analysis was 93% DM, 65% CP, 8% ash, 0.5% crude fat and 1% fiber. The calculated AMEn for Spirulina was 2,656 kcal/kg (DM basis), and apparent ileal digestibility coefficients were: 70.4% Lys; 68.5% M + C; 71.3% Thr. There was no statistical impact on performance parameters, processing yields, fat pad or breast myopathies from increased inclusion of Spirulina in 49d broilers. There was however a 9-point increase in FCR when algae inclusion reached 3%. Redness ($P=0.011$) and yellowness ($P < 0.001$) in the pectoralis major increased linearly with Spirulina inclusion. The performance results validate the calculated digestibility coefficients. The linear increase in breast meat color indicates that pigmentation will continue to increase as larger rates of algae are included. Spirulina can be formulated into broiler diets at low inclusion levels with minimal impacts on performance and can provide increases in carcass pigmentation.

Key Words: Spirulina, digestibility, broilers, pigmentation, formulation

207 Energy expenditure in broiler chicks under health challenges. Rosiane D. Camargos*^{GS}, Rony Riveros Lizana, Guilherme F. Teofilo, Luis Filipe V. de Freitas, Bruno Balbino Leme, Nilva Sakomura; *Animal Science, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil*.

Understanding the mechanism of energy expenditure under a health challenge in birds to supply the energy and attenuate the effects of the sanitary problems is essential. This study aimed to evaluate the daily energy expenditure in growing broiler chicks under health challenges. Forty-eight day-old male Ross 308 broiler chicks 13-day-old were allocated into open-circuit chambers. The birds were randomly distributed in two treatments (Challenge with *E. maxima* and *C. perfringens* - C and no challenge pair-feeding group - PF), with three replicates of eight birds, each with the same body weight. On the 14-old-day, the birds of the challenged treatment were inoculated with 1ml of *Eimeria maxima* (7×10^3 oocysts/ml) by oral gavage. On days 18, 19, and 20 was inoculated 2.5×10^6 CFU of *Clostridium perfringens*. The PF group was inoculated with 1 ml of saline solution. The gas exchange was measured daily during the feeding condition from 14 to 22-

old-day. Also, was measured body weight (BW) and the feed intake (FI) to calculate the feed efficiency (FE). The heat production (HP) was obtained from the oxygen consumption (VO_2) and carbon dioxide production (VCO_2), using the Brouwer (1965) equation, and the respiratory quotient was obtained from VCO_2/VO_2 ratio. The data was analyzed by ANOVA two-way considering each day as a longitudinal factor, using Minitab v.20 statistical software. The FI was similar between treatments, for the FE was shown an interaction between treatment and age ($P < 0.01$). The difference ($P < 0.01$) was shown on the first day before the *E. maxima* were inoculated (14, 15, and 16). The PF report high values than C treatment ($P < 0.01$) during all observation periods. The birds of the C group had a lower BW compared to PF ($P < 0.001$). Challenge birds had a significantly higher HP ($P = 0.013$) than PF treatment, with a difference of 32.1 KJ/kg/0.75/d, but no interaction was observed. Also, the RQ was not different between treatments ($P = 0.593$), but it was affected by the longitudinal factor ($P < 0.001$) and was shown interaction ($p = 0.034$). During the challenge period (between 18-21 d), RQ was higher than 1 in challenged birds. In conclusion, a moderate health challenge affects feed efficiency utilization and increases energy expenditure, representing energy loss.

Key Words: *Clostridium perfringens*, *Eimeria maxima*, energy expenditure, growing birds, heat production

208 Evaluation of semi-purified diets for glucose recovery in order to estimate endogenous losses of energy in broiler chickens. Stephanie Philpot*^{GS 1}, Aaron Cowieson², William A. Dozier, III¹; ¹*Poultry Science, Auburn University, Auburn, Alabama, United States*, ²*DSM Nutritional Products, Kaiseraugst, Switzerland*.

Standardization of metabolizable or digestible energy for basal endogenous losses of energy (EEL) may reduce variability of energy assays or increase assay sensitivity. Two experiments were designed to evaluate suitability of diets for estimating EEL by evaluating presence of glucose in the terminal ileum. In both experiments, day-old YP × Ross 708 male chicks were placed into 32 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 weighed and provided with semi-purified experimental diets. In experiment 1, diets primarily consisted of dextrose, dextrose and cellulose, dextrose and starch, or dextrose, starch, and casein. In experiment 2, diets were dextrose-based with 5% cellulose and 0, 5, 10, and 15% casein. All diets contained titanium dioxide as an indigestible marker and included supplemental vitamins and minerals to balance nutrients and dietary electrolyte balance. Excreta were collected quantitatively at 21 and 22 D of age and birds and feed were weighed at 22 D of age. Birds were necropsied for collection of duodenal, jejunal, and upper and terminal ileal digesta at 23 D of age. Glucose recovery, titanium dioxide concentration, and gross energy were determined in excreta and terminal ileal digesta samples. Glucose content was analyzed in all digesta samples. Each experiment was

conducted as a randomized complete block design with 8 replicates per diet. Growth performance characteristics and EEL were analyzed with one-way analysis of variance. Additionally, preplanned orthogonal contrasts were utilized in experiment 1, and regression analysis was utilized in experiment 2. Glucose presence in the terminal ileum was analyzed as a one-sample one-tail T-test where $H_0 = 0$. In experiment 1, birds provided with the dextrose diet had insufficient digesta for analysis, while glucose was recovered in ileal digesta of chicks provided diets containing starch ($P \leq 0.0171$). Chicks fed the diet containing casein had increased BW gain compared with chicks fed the dextrose and cellulose diet (8 g vs. -50 g, $P < 0.0001$). No glucose was recovered in terminal ileal digesta in chicks provided with any diet in experiment 2 ($P \geq 0.09$). Body weight gain was maximized in chicks provided with 10 or 15% casein ($P < 0.0001$). Casein inclusion rate did not affect EEL ($P = 0.38$). These experiments demonstrate that broilers have a measurable basal EEL which may affect additivity of ingredients in diet formulation. Additionally, these experiments indicate that a dextrose-based semi-purified diet with 5% cellulose and 10% casein is appropriate for estimating EEL in broilers.

Key Words: Assay, Broiler, Endogenous loss, Energy, Semi-purified diet

209 Effect of beak trimming at hatch and the inclusion of oat hulls in the diet on water intake, preference behavior for coarse particles, and gastrointestinal tract traits of brown-egg pullets from 0 to 5 weeks of age. N. L. Corrales*^{GS}, J. Ben Mabrouk, N. García, A.F. de Juan, Gonzalo Mateos, L. Cámara; *UPM, Madrid, Madrid, Spain*.

The effects of infrared beak trimming (BT) at hatch and the inclusion of oat hulls (OH) in the diet on water intake, preference behaviour for coarse particles, and the development of the gastrointestinal tract (GIT) were studied in brown-egg pullets from 0 to 5 wk of age. The experimental design was completely randomized with 4 treatments arranged as a 2×2 factorial with 2 beak treatment procedures (intact beak vs. beak trimming) and the inclusion or not of 3% OH in the diet as main effects. Each treatment was replicated 20 times and the experimental unit was a cage with 10 pullets. The control diet was based on corn and soybean meal and contained 2,960 Kcal AMEn/Kg, 19% CP and 0.98% digestible Lys. Water to feed intake ratio was determined by week and cumulatively. In addition, the preference for consuming coarse particles was measured at 4 wk of age as the difference of the geometric mean diameter (GMD) and the geometric standard deviation (GSD) between the original feed at the start of the experiment and the residuals 24 hours after feed provision. Immediately after the last control (5 wk of age), two birds per replicate were selected randomly, sacrificed, and used to measure GIT development. Data were analysed as a completely randomized design using the MIXED procedure of SAS with BT and OH inclusion as main effects and their interaction. When the data was

significant, the Tukey test was used to separate treatment means. From 0 to 5 wk of age, no interactions between beak trimming and OH inclusion were detected for any trait, and therefore, only main effects are presented. Water intake and water to feed intake ratio were greater for the non-treated than for the beak-trimmed pullets ($P < 0.001$). The preference for coarse particles was greater for the intact beak birds than for the beak-trimmed birds ($P < 0.001$). Diet did not affect any of the GIT traits studied ($P > 0.05$). An interaction between beak trimming and age was observed for water intake and water to feed intake ratio. Non-treated pullets drank more water and had higher water to feed intake ratio than beak-trimmed pullets from 0 to 4 wk of age. However, no differences were detected thereafter. In summary, infrared beak trimming at hatch reduced water intake and water to feed intake ratio but did not affect GIT development. Non-trimmed pullets showed a clear preference for coarse particles compared with treated pullets as indicated by the higher percentage of fine particles ($< 315 \mu\text{m}$) in the residual feeds. The inclusion of OH in the diet did not affect water intake or GIT development but increased the preference of the pullets for coarse particles ($P < 0.001$).

Key Words: beak trimming, oat hulls, preference behaviour, pullets, water intake

210 Feed processing technologies on *Camelina sativa* and its effect on performance in laying hens. Kiana A. Rieger*^{GS}, Rex Newkirk; *Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.*

Incorporating *Camelina sativa* (camelina) meal into poultry feed provides an alternative ingredient to flax in order to produce an omega-3 egg. However, anti-nutritional factors limit the inclusion level to 10% in laying hen formulations, reducing its efficacy for use in omega-3 egg production. It was hypothesized that heat treating camelina would reduce anti-nutritional factors and increase feed efficiency and egg production, and that cold-pressed camelina meal would result in similar egg production and feed efficiency to flax meal. A total of 1,080 Lohmann LSL Lite laying hens were fed one of ten experimental treatments ad libitum in a 112-day trial consisting of a 5x2 factorial design including five experimental meals (extruded camelina, pelleted camelina, cold-pressed camelina, hot-pressed camelina, flax) by two inclusion levels (10% and 20%). Each treatment was replicated 9 times, with 12 birds per replication. Experimental diets were formulated to reach the omega-3 polyunsaturated fatty acid composition required to produce an omega-3 claim ($>300 \text{ mg/egg}$). Hen performance was measured through feed consumption, egg production, and visual assessment of the eggs. Egg production was assessed 5 days a week and analysed as percent lay, visual assessment was recorded during daily egg collections, and feed was weighed back every 28 days. The data was analysed as a 2-way ANOVA in a completely randomized block design as a 5x2 factorial with statistical significance being declared at

$P < 0.05$. The 20% flax meal treatment was terminated after 4 weeks of the study due to excessive reductions in body weight and egg production. At 10% inclusion, flax meal and cold-pressed camelina meal resulted in the lowest average percent lay for the overall period (84.4% and 83.3%, respectively). Hot pressing and pelleting camelina meal numerically increased production over both flax and cold-pressed camelina meal (91.4% and 90.7%, respectively). Flax meal resulted in the highest feed intake but the lowest egg production. However, pelleting and hot pressing tended to increase feed intake suggesting that reductions in anti-nutritional factors may have improved palatability. At the 20% level, egg production decreased in all treatments. Extrusion and pelleting numerically increased production over hot and cold-pressed camelina meal (83.9%, 80.4%, 72.6% and 76.4%, respectively). There were no significant differences in feed intake at the 20% level. In conclusion, heat processing camelina improved the performance of laying hens through an increased egg production. However, at the 20% inclusion level, performance was decreased across all feed groups.

Key Words: camelina, flax, feed processing, fatty acids, omega-3

211 Influence of the inclusion of *Aspergillus oryzae* on productive performance and egg quality of brown hens from 15 to 43 weeks of age. A.F. de Juan*^{GS}¹, Ignacio R. Ipharraguerre², J. Ben Mabrouk¹, C. Ocasio-Vega³, L. Aguirre¹, Gonzalo Mateos¹; ¹UPM, Madrid, Madrid, Spain, ²Institute of Human Nutrition and Food Science, University of Kiel, Kiel, Germany, ³BioZyme Incorporated, St. Joseph, Missouri, United States.

The object of this study was to determine the effects of the dietary inclusion of either a prebiotic (PRE) or a postbiotic (PTO) both of them obtained from *Aspergillus oryzae* (AO) on performance and egg quality traits of Lohmann Brown Classic hens from 15 to 43 wk of age. The experimental design was completely randomized with 3 treatments that consisted in a control diet with 2,750 kcal AMEn/kg and 16.9% of CP and the same diet supplemented with 50 ppm of PTO or 500 ppm of PRE. Each treatment was replicated 24 times and the experimental unit was the cage with an individual hen. The experiment lasted 28 wk (7 periods of 4 wk each). Egg production, egg weight, shell-less, broken, and dirty eggs, and mortality were controlled daily, and feed disappearance was measured weekly. The proportion of albumen, yolk, and shell of the eggs, shell quality traits (weight, strength, and thickness), and Haugh units were measured in all the eggs produced for the last 2 d of each of the 7 experimental periods. BW of the hens was measured at the start of the trial and at the end of each of the 7 experimental periods. In addition, the quality of the excreta (moisture content and visual score by two independent observers) and the AME of the feeds were determined at 35 wk of age. Also, *Escherichia coli*, *Clostridium perfringens*, total coliforms, Enterobacteriaceae, and *Lactobacillus* spp. colonies were

measured in representative excreta samples. Data were analyzed as a completely randomized design with type of diet as main effect by using the GLM procedure of SAS. In addition, the effect of AO supplementation was also studied by using orthogonal contrast (AO containing diets vs. control diet). Contrast of AO containing diets against the control diet was done by using a Dunnett's test. From 15 to 43 wk of age, AO supplementation reduced ($P < 0.05$) the incidence of shell-less eggs. In addition, from 15 to 21 wk of age, the feeding of PTO increased egg production by 9% and improved FCR by 8% but the differences were not significant. No significant effect was observed for any of the bacteria colonies studied. The inclusion of AO in the diet might increase hen performance and eggshell quality. A second study to measure hen production and egg quality during the first stages of the egg cycle using a larger number of hens to reduce the variability among replicates is granted.

Key Words: *Aspergillus oryzae*, egg quality, hen performance, laying hen, postbiotic

212 Allometric coefficient and energy for maintenance in pullets and laying hens. Freddy Horna*^{GS}, Matheus D. Reis, Rony Riveros Lizana, Raully L. Silva, Carolina Cardoso Nagib Nascimento, Bruno Balbino Leme, Nilva Sakomura; *Animal Science, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil.*

In the net energy system, the relationship between basal metabolic rate (or fasting heat production, FHP) and body mass (BW) is an important issue in energy requirements estimation. FHP is typically scaled with BW according to the equation $FHP = aBW^b$, where a is the constant and b is the allometric exponent. The allometric coefficient of 0.75 suggested by Kleiber is commonly accepted for many species; however, different allometric coefficient was suggested for growing pigs (0.60), growing calves (0.85), and growing turkeys (0.70). These differences in allometric coefficient can lead to under or overestimations of energy required for maintenance. The purpose of this study is to reassess FHP in modern pullets and laying hens and determine a specific exponent for expressing the metabolic BW. A total of forty-eight observations of FHP were obtained in different groups of white lohmann birds with body weight ranging between 0.08 and 1.60 kg. Birds were fed ad libitum before fasting. Heat production was measured using indirect calorimetry in two calorimetry chambers (0.9m x 0.9m X 0.85m). Following bird chamber placement, birds were fed for three days (adaptation period), then, the feed was removed at the beginning of the fasting period. All birds had continuous access to water during the fasting period. In each run, the number of birds was reduced according to chamber size. Concentrations of O_2 and CO_2 entering and exiting the calorimetry chambers were recorded two times per hour. Oxygen consumption (vO_2) and carbon dioxide production (vCO_2) were estimated as the difference between incoming and outgoing chamber gases, multiplied by the chamber airflow rates. Brouwer's equation (1965) was subsequently used to

estimate FHP from the vO_2 and vCO_2 values. FHP was standardized as the plateau or asymptotic heat production following a fast of at least 8 hour. Recorded data of FHP and BW were logarithmically transformed to fit linear regression of the form $\log(FHP) = \log(a) + b \cdot \log(BW)$, where a is the constant term and represents FHP (kcal/BW^b), and b is the allometric coefficient. The allometric coefficient estimated was $b = 0.67$ (95% confidence limits: 0.648-0.696), while the estimated FHP was $a = 90.1$ kcal/BW^{0.67} (95% confidence limits: 88.2-91.9). The confidence limits of allometric exponent include 0.67 and excluded 0.75 exponent. In conclusion, the value of 0.67 was found to be appropriate to describe the ratio of metabolic body weight and 90.1 kcal/BW^{0.67} was estimated to be the amount of energy to meet the maintenance needs for pullets and laying hens.

Key Words: fasting heat production, indirect calorimetry method, net energy

213 Withdrawn.

214 Metabolomics biomarkers of sexual development in broiler breeders. Mohammad Afrouziyeh*^{GS 1}, Nicole Zukiwsky², Douglas R. Korver², Martin J. Zuidhof²; ¹University of Alberta, Edmonton, Alberta, Canada, ²University of Alberta, Edmonton, Alberta, Canada.

Profiling the plasma metabolome provides a new perspective for studying the metabolic aspects of sexual maturity in broiler breeder hens, a better understanding of its biological mechanisms, and provides potential biomarkers for predicting sexual development. The objectives of the current study were to evaluate the effect of maturity (pullet vs. hen) and onset of lay (early vs. late) on plasma metabolome dynamics to identify potential biomarkers that predict sexual development. A total of 36 pullets were used, 30 of which were randomly assigned to one of 10 unique growth trajectories, and 6 were not feed restricted. The growth trajectories were designed using a 3-phase Gompertz growth model with 10 levels of BW gain in the prepubertal and pubertal growth phases ranging from the breeder-recommended target BW to 22.5% higher, in 2.5% increments. The BW trajectories were applied using a precision feeding system, which collected BW and feed intake data for each individual bird. Birds were classified post hoc based on age at first egg (AFE), and 12 pullets were chosen from the lower and upper AFE extremes (early and late onset of lay) for repeated plasma metabolomic assays (collected at 18, 20, 22, 24, and 26 wk of age) using a direct-injection liquid chromatography-tandem mass spectrometry and steroid assays. Univariate analysis identified 87 differential metabolites between the early and late onset of lay groups at 24 wk of age and 104 differential metabolites between the pullet and hen groups. We identified 15 potential biomarkers to predict pullet to hen transition by analyzing receiver operating characteristic curve, mainly consisting of carnitine and choline metabolites. Differential

metabolites during the pullet to hen transition were mainly associated with lipid, energy, and amino acid metabolism pathways. At 24 wk of age, the main pathways involved in differentiation of the early- and late-onset of lay groups were related to lipid and amino acid metabolism, which could be involved in biosynthesis of egg yolk precursors in the liver. In the current study, increasing prepubertal and pubertal BW gains by more than 15% of the breeder-recommended target BW triggered fat metabolism and yolk precursor synthesis, which consequently advanced sexual maturity. In conclusion, this study indicated that metabolic transition during the onset of lay in broiler breeders is accompanied by certain metabolic signatures that can be used to predict the metabolic status linked to the bird's maturity.

Key Words: broiler breeder, onset of lay, metabolomics, metabolic status, maturation

215 Understanding factors that affect soybean meal quality. Muhammad Ali*^{GS 1}, Gustavo Quintana-Ospina¹, Maria C. Alfaro-Wisaquillo¹, Danny B. Patino¹, Michael Joseph¹, Rachel Vann², Edgar O. Oviedo-Rondon¹; ¹*Prestige Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, United States*, ²*Crop and Soil Sciences, North Carolina State University, Raleigh, North Carolina, United States*.

Nutrient and trypsin inhibitor (TI) content of soybeans can be influenced by genetics, planting site, and maturity group, among other factors. Heat treatments during soybean processing reduce most of TI, but the initial content of TI and other thermoresistant antinutritional factors affect soybean meal quality for use in poultry feed. In this study, we assessed the CP, Lys, Met, Thr, and Trp content (%) as well as TI (mg/g) in four commonly grown varieties of soybeans (AG56X8, AG69X0, S53-F7X, and S62XT09, labeled here A to D, respectively) planted in 3 locations of North Carolina (counties Robeson, Beaufort, and Rowan, labeled as X, Y, Z, respectively) in two maturity groups double crop (growing two successive crops in one year) and full season (growing one traditional crop in a year). The 4 x 3 x 2 factorial arrangement of treatments resulted in 24 treatment combinations. A completely randomized design with four plot replicates was used. Raw soybean samples were cleaned from foreign material and standardized to the USDA soybean grading system to be grade 3 or 2. These samples were either submitted to wet chemistry by AOAC methods or ground in a Retsch grinder at 16000 rpm with a sieve opening of 0.5 mm to be analyzed by NIRS. NIRS spectra were collected in a FOSS DS 2500, and data were read with the Soya package AMINONIR[®] RED (Evonik). Data were analyzed using a three-way ANOVA while mean separation was done by Tukey's or student's t-test. All data were standardized to dry matter content before statistical analysis. Results by wet chemistry and NIRS gave similar results ($P < 0.001$) with a strong correlation between both methods ($r = 0.99, 0.97, 0.71, 0.97, 0.88, \text{ and } 0.90$ for CP, Lys, Met, Thr, Trp, and TI, respectively). Results of both

datasets indicated similar three-way interaction effects ($P < 0.01$). Soybean varieties B and C in location X had higher CP, Lys, Met, Thr, and Trp content than crops of Y and Z locations, and results did not vary according to maturity group. The highest TI content was observed in variety D (S62XT09) and the lowest in variety A (AG56X8). In general, beans from full season had higher nutrient content and lower TI than those from double-crop. The lowest CP and amino acid content and the highest TI were observed in variety D (S62XT09) in Y and Z locations in both maturity groups. There was ($P < 0.001$) a negative correlation (average $r = -0.75$) between TI and nutrient content, except for Met ($P > 0.05$). In conclusion, soybean variety, plantation location, and maturity group may influence the soybean nutrient profile and TI activities. Better understanding and tracking of these agronomic factors may help to improve soybean meal quality and reduce its variability.

Key Words: Soybean Quality, Amino Acids, Trypsin Inhibitor, Soy Genetics, Maturity Groups

216 Effects of soybean meal particle size on feed milling efficiency, pellet quality, broiler performance (D1-42) and processing characteristics. Alyssa Lyons*^{GS}, Logan S. Erb, Courtney Poholsky, Paul Patterson, John W. Boney; *Animal Science, Penn State University, University Park, Pennsylvania, United States*.

Ingredient particle size (PS) is known to affect pellet quality (PQ). The literature supports improved performance when PQ is improved. Recent research indicated that ingredient PS also affects amino acid (AA) digestibility. The objectives of this study were to determine the effects of soybean meal (SBM) PS on feed milling efficiency, PQ parameters, broiler performance, and processing characteristics. Dietary treatments were created by reducing SBM PS with three hammermill screens (3/32" (2.4 mm), 7/32" (5.6 mm), and 10/32" (7.9 mm)). These screens represent the treatments used in each experiment. Experiment 1 was conducted at the pilot feed mill at West Virginia University using the finisher period feed that was provided in experiment 2. Experiment 1 was arranged in a 3 x 3 Latin Square design with three 453.6 kg replicate batches per treatment. Hot pellet temperature and production rate were collected in duplicate and provided as an average. PQ analyses were conducted 24-h post-pelleting. In experiment 2, 1,152-day-old male HR708 broilers were housed in 12 replicate floor pens per treatment. Treatments were arranged in a randomized complete block design with a pen of 32 birds serving as the experimental unit. Birds were fed one of three treatment diets varying in SBM PS (3/32", 7/32", or 10/32") from d1-42 in a three phase (starter, grower, finisher) feeding program. Birds and feed were weighed to calculate FI, LWG, and mortality corrected FCR at the end of each feeding phase. On d42, two broilers per pen were individually weighed, stunned, exsanguinated, and slaughtered to determine processing yields and gizzard

weights. All data were analyzed using the GLM procedure of SAS using a one-way ANOVA. The F-protected Fisher's LSD test was used to separate means when $P \leq 0.05$. In experiment 1, SBM PS did not affect hot pellet temperature or production rate ($P > 0.05$). However, PQ improvements were apparent when the small SBM (3/32" screen) was used ($P < 0.05$). It is noteworthy that small SBM did cause the pellet die to plug during each manufacturing replicate. In experiment 2, d1-42 broiler performance demonstrated higher FI, LWG, and FCR when the small SBM (3/32" screen) was provided compared to when larger SBM (7/32" and 10/32" screens) was provided. These data support previously reported findings of reduced AA digestibility when small SBM is provided to broilers. FI likely increased to meet AA needs, providing additional nutrients for maximal growth. However, FCR was negatively impacted. Finally, SBM PS did not affect processing yields ($P > 0.05$). These data indicate that SBM PS does impact feed mill efficiency, PQ, and broiler performance parameters when fed in a pelleted diet.

Key Words: hammermill, soybean meal, particle size, pellet quality, feed mill

217 Variability in particle size of different types of soybean meal subject to different roller mill settings.

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Soybean meals are the most widely used source of high-quality vegetable protein for livestock, aquaculture, and especially for poultry feed. It may be processed in a variety of different ways to increase the protein concentrations and to deactivate anti-nutritional factors. The quality of soybean meal (SBM) is affected by nutrient and antinutritional content, as well as physical characteristics with one being particle size (PS). The PS is an important factor of feed manufacturing that impacts mixability, feed flowability, pellet quality, and animal performance. The objective of this study was to evaluate the effects of grinding four different SBM types (Full-fat, FFSBM; Solvent-extracted SBM, SESBM; Expeller-extruded SBM, EESBM; and High-oleic SBM, HOFFSBM) at different roller mill gap settings on PS. These four sources of SBM were milled using a two-pair roller mill (Model C128889, RMS, Sea, SD) at 10 different settings: 0-0, 0-5, 15-10, 15-15, 20-20, 30-25, 35-20, 40-35, 50-25, and 50-50 (top pair-bottom roller pair settings). The PS of three replicate samples per roller-gap settings for each type of SBM were analyzed using the ANSI/ASAE S319.4 "Method of determining and expressing fineness of feed materials by sieving" standard using 13 sieves and an electric sieve shaker (ANSI) accompanied by 0.5 g of silicone dioxide dispersion agent and sieve agitators. The weight of particles retained by each sieve was expressed as a percentage of the total sample recovered. The average PS of the samples was expressed as the geometric mean diameter (d_{gw}) and standard deviation

(S_{gw}). Data were analyzed using one-way ANOVA and means were separated using Tukey's test or student's t-tests. Additionally, a regression analysis was conducted. These settings generated samples with average d_{gw} of 574, 623, 681, 732, 778, 858, 881, 892, 905, and 947 μm , respectively. Regression analysis indicated negative quadratic effects ($P < 0.001$) of roller gap on d_{gw} with R^2 of 0.83, 0.92 and 0.90 for FFSBM, HOFFSBM, and SESBM, respectively. These effects were observed in all the meals except EESBM which had ($P < 0.001$) a linear effect ($R^2 = 0.79$). The SESBM had the largest particle size followed by FFSBM, HOFFSBM, and EESBM. However, both FFSBM and HOFFSBM broke with similar d_{gw} ($P < 0.001$). The S_{gw} decreased ($P < 0.001$) linearly for EESBM ($R^2 = 0.73$), FFSBM ($R^2 = 0.73$), and HOFFSBM ($R^2 = 0.83$) as the roller-gap was reduced. The S_{gw} was lower for the FFSBM and HOFFSBM than the SESBM and EESBM. In conclusion, the d_{gw} and S_{gw} of the SBM varies when ground under the same gap-settings in a roller mill depending on the type SBM and potentially fat.

Key Words: Soybean meal, full-fat soybean meal, particle size, roller mill

218 Effect of different soybean meal inclusions in broiler diets on the Arkansas Net Energy, performance and body composition from d0 to d56 of age.

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Arkansas Net Energy (Ark NE) is a NE system for ingredients, diet and feed additives that provides actual productive energy (PE) and is dependent upon genetics, age, environment and nutrient content. The advantage of utilizing Ark NE instead of AMEn and Classic NE is the Ark NE system measures actual net energy utilized for maintenance (NE_m) and gain (NE_g). Broilers need a dietary energy system that reflects performance, high protein gains and body composition consisting of a high percentage of protein mass. Soybean meal (SBM) is an important protein source for broiler diets because it contains high quality protein with excellent amino acid digestibility. Diets with increasing SBM inclusions were used to determine the Ark NE, performance, and body composition of broilers for 56 days using dual energy x-ray absorptiometry (DEXA) and indirect calorimetry. A total of 2,616 fast-growing male broilers were randomly assigned to 96 floor pens for 56 day feeding study with 5 dietary phases. In each dietary phase, broilers were given a corn-soybean meal basal diet formulated with three different inclusion levels of SBM to determine the Ark NE and nutritive value of experimental diets. Data were subjected to a one-way ANOVA and Tukey's HSD to compare separate treatment means with significance set at 0.05 using JMP pro 15.2 (SAS Institute, 2018). A linear regression model was used to establish the Ark NE value of diets and SBM. Using the Ark NE equation, broiler performance including protein and fat mass gain was higher ($P < 0.05$) for broilers fed the highest

SBM level compared to lower SBM inclusions in each feeding- phase. For each gram increase in SBM intake/broiler, the predicted broiler protein mass increased by 0.43, 0.38, 0.36, 0.47 and 0.25 grams, and the predicted broiler fat mass increased by 0.02, 0.19, 0.19, 0.12, and 0.09 grams in starter, grower, finisher I, II and III, respectively. Consequently, the Ark NE values of SBM were 1401.32, 1950.75, 2046.27, 2140.66 and 2718.26 kcal/kg in starter, grower, finisher I, II and III. Additionally, Ark NE: AMEn ratios of SBM were 68.29, 92.89, 99.51, 105.32 and 133.68 in starter, grower, finisher I, II and III, respectively. The AMEn system underestimated actual productive energy (Ark NE) for SBM by 462 kcal/broiler during 56 day feeding study.

Key Words: Broilers, Soybean meal, Net Energy, DEXA, Indirect Calorimetry

219 Chemical composition, protein quality, and in vitro protein digestibility of commercial soybean meals from different origins. L. Aguirre*^{GS 1}, A. Smith², J. J. Arroyo³, A.F. de Juan¹, G. Fondevila¹, H. Kadardar¹, Gonzalo Mateos¹, L. Cámara¹; ¹UPM, Madrid, Madrid, Spain, ²DSM Nutritional Products (UK) Ltd, Heanor, Derbyshire, United Kingdom, ³DSM Nutritional Products Ltd, Madrid, Alcalá de Henares, Spain.

An experiment was conducted to study the influence of the origin of the beans on the chemical composition, protein quality, and nutritive value of the corresponding soybean meals (SBM). In addition, the in vitro protein digestibility of the meals was determined using a two-step test that simulated the digestive processes occurring in the gastrointestinal tract of the chick. A total of 160 commercial samples of SBM from South Africa (SAF), Argentina (ARG), Brazil (BRA), and USA, in equal numbers, were collected by specialized quality control personnel. Samples from SAF were collected in the country of origin, whereas the samples from the other 3 origins were obtained from crushing plants in different European locations or at the arrival of the vessels to European ports. The data were analyzed as a completely randomized design using the GLM procedure of SAS with country of origin of the SBM as main effect. When significant differences were detected, the Tukey test was used to separate treatments means. On 88% DM bases, the BRA meals had more crude protein and crude fiber contents than all the other meals ($P < 0.01$). The highest ether extract (EE), sucrose, and phosphorus content were observed for the SAF meals ($P < 0.01$). The amino acid (AA) profile of the SBM varied with the origin of the beans, with the SAF and USA meals having more Lys and total sulfur AA per unit of protein than the South American SBM ($P < 0.01$). In general, all protein quality indicators [urease activity (UA), protein dispersibility index (PDI), KOH solubility (KOH), and trypsin inhibitor activity (TIA)] were lower for the ARG meals than for all the other meals ($P < 0.01$). The in vitro protein digestibility, determined in 30 SBM samples per origin after 3 h of incubation, was higher for the SAF than for the ARG and BRA meals with

the USA meals being intermediate ($P < 0.01$). Independently of SBM origin, in vitro protein digestibility values were highly correlated with TIA ($r = 0.442$; $P < 0.001$) and KOH ($r = 0.234$; $P < 0.05$) but not with UA or PDI. Based on accepted prediction equations, the AMEn_n content of the SBM was higher ($P < 0.001$) for the SAF meals than for the South American meals with the USA meals being intermediate. It is concluded that the origin of the beans affects the chemical composition, protein quality, and in vitro protein digestibility of the SBM. Because of their higher EE and sucrose content, the AMEn_n of the SBM was greater for the SAF meals than for the USA meals and greater for both than for the South American meals, although the differences not always were significant. In summary, feed mill managers should use different matrices to estimate the chemical composition and nutritive value of SBM of different origins.

Key Words: amino acid profile, in vitro N digestibility, protein quality indicator, proximal analyses, soybean origin

220 Effects of varying corn particle size on broiler performance and organ development from 1 to 21 days of age. Emily K. Stafford*^{UG1}, Gabrielle Harder¹, Kevin M. Downs¹, Joseph P. Gulizia², Santiago J. Sasia², Wilmer J. Pacheco²; ¹School of Agriculture, Middle Tennessee State University, Murfreesboro, Tennessee, United States, ²Department of Poultry Science, Auburn University, Auburn, Alabama, United States.

This research evaluated the influence of varying dietary corn particle size (CPS) on body weight, feed consumption, and feed conversion of broilers grown to 21 days of age. Previous research has demonstrated that CPS can influence feed costs, nutrient digestibility, and potential profitability. Broiler starter diets with CPS of either 832, 1432, or 2036 μm were fed to 288 male Cobb 700 broilers over a 21-d battery cage grow out (6 replicate cages per treatment; 96 birds per treatment). Bird weights and feed consumption data were collected at 7 d intervals. At study termination, 6 birds per cage were euthanized for organ data collection (crop, proventriculus, gizzard, and ceca). Data were analyzed as a completely randomized design, with battery cage representing the experimental unit, using the GLM procedure of SAS. Means were separated using Tukey's HSD and statistical significance considered at $P \leq 0.05$. There were significant treatment effects for average body weight at d 7 ($P = 0.050$), with birds on the 832 μm CPS diet weighing more (180.3 g) than birds on 1432 and 2026 μm diets (176.5 and 169.0 g, respectively). There were, however, no CPS effects on BW at d 14 and 21 ($P > 0.05$). Overall, there were no significant treatment effects for feed consumption d 0 to 7 ($P = 0.429$), 0 to 14 ($P = 0.511$), or 0 to 21 ($P = 0.939$); and feed conversion d 0 to 7 ($P = 0.832$), 0 to 14 ($P = 0.252$), or 0 to 21 ($P = 0.189$). Additionally, there were no observed treatment differences for mortality anytime during the 21 d grow out ($P > 0.05$). Corn particle size did not influence relative (g/kg BW) crop, gizzard, or ceca weights. However, birds on 832 μm CPS had 14 and 13%

larger ($P < 0.05$) relative proventriculus weights respective to the 1432 and 2036 μm treatments. Varying corn particle sizes did not significantly impact most performance and organ parameters during the broiler starter phase; however, based on these results 832 μm CPS may be recommended for higher bird weights and a more developed proventriculus to improve digestibility.

Key Words: broiler, corn particle size

221 Effects of fiber type, particle size, and inclusion level on the growth performance, digestive organ growth, intestinal viscosity, intestinal morphology, and gene expression of broilers. car J. Tejada*^{GS}, Woo K. Kim; *Poultry Science, The University of Georgia, Athens, Georgia, United States.*

Inclusion of cheap and fibrous feed ingredients is gaining attention in the poultry industry, which makes the understanding of the nutritional and physiological role of dietary fiber of importance. Therefore, the objective of this study was to evaluate how fiber type, particle size, and inclusion level affect the growth performance, organ development, intestinal viscosity, intestinal morphology and gene expression in broiler chickens. A total of 648 one-day old Cobb® male broilers were randomly assigned to a control diet and 8 other dietary treatments divided in 2 fiber types (cellulose vs soyhulls), 2 particle sizes (100 and 600 μm), and 2 inclusion levels (4% and 8% crude fiber). Birds were reared to 21 days of age in battery cages ($n=6$ replicates). On day 21, digestive organ weights were recorded for analyses of organ growth, ileal digesta samples were taken for analyses of intestinal viscosity, intestinal samples were taken for analyses of histology, and jejunal mucosas were collected for analyses of nutrient transporters. Data were analyzed as a completely randomized block design with 8 treatments organized as $2 \times 2 \times 2$ factorial using JMP® 2021. Orthogonal contrasts were used to determine the effect of fiber inclusion compared to the control group or individually, whereas the main effect interactions were evaluated excluding the control group to be able to assess the effect of the independent variables without the variability introduced by the control group. Groups fed 8% crude fiber from cellulose (8% CL) had the lowest weight gain regardless of the particle size ($P < 0.01$). The control group had the highest feed intake among treatments ($P < 0.01$). Groups fed 8% crude fiber from soyhulls (8% SH) with a coarse particle size had the heaviest relative gizzard weight among treatments ($P = 0.045$). Groups fed 8% SH had the heaviest small intestine weights regardless of the particle size ($P = 0.009$). No differences were observed in the relative weights of the liver and ceca. The highest viscosity was observed in the group fed 8% SH with a fine particle size ($P < 0.001$). The group fed 4% SH with a coarse particle size had the longest duodenal villus ($P < 0.001$). The shortest jejunal villus height was observed in the group fed 8% CL with a fine particle size ($P < 0.001$). Ileal villus was highest in groups fed high cellulose levels regardless of the particle

size ($P < 0.001$). The highest expression of peptide transporter 1 was observed in the group fed 8% CL with a coarse particle size ($P = 0.008$). In conclusion, fiber type, particle size, and inclusion levels are important factors in the regulation of intestinal morphology, viscosity, nutrient transporters, and growth performance.

Key Words: fiber, particle size, inclusion level, intestinal morphology, broiler

222 Effects of improvements to pellet quality on commercial hen turkey performance and processing parameters. Courtney Poholsky*^{GS}¹, Morgan L. Watt¹, Logan S. Erb², Alyssa Lyons³, John W. Boney⁴; ¹*Penn State University, Howard, Pennsylvania, United States*, ²*Animal Science, Penn State, State College, Pennsylvania, United States*, ³*Animal Science, Pennsylvania State University, State College, Pennsylvania, United States*, ⁴*Animal Science, Penn State University, University Park, Pennsylvania, United States.*

Performance benefits of feeding improved pellet quality (PQ) are well documented for broiler chickens. However, the effects of feeding improved PQ feed to modern commercial turkeys needs revisited. Proudfoot and Hulan suggested that turkeys may be more sensitive to PQ than broilers; however, this was suggested nearly 40 years ago. The study objective was to investigate the effect of PQ on weeks 5-16 hen turkey performance and processing variables. Poor pellet quality (PPQ) feed and improved pellet quality (IPQ) feed were provided to eight replicate pens of Nicholas Select hen turkeys from weeks 5-16. Each of the 16 pens contained 28 hen turkeys, totaling 448 birds. A 7-phase feeding program followed Aviagen nutrient recommendations. A common Starter 1 crumble was fed to poult from weeks 1-4. After ensuring week 4 body weights were similar across all pens, hens were provided nutritionally identical diets differing only in PQ. The IPQ feed was manufactured at a commercial feed mill using techniques known to create 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 chosen 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 the initial PPQ allotment were ground using a hammer mill, creating fines. Calculated amounts of pelleted feed and fines were then mixed to reduce the P:F of the IPQ feed by 30% for each phase. IPQ feed averaged 89.8% pellets while PPQ feed averaged 59.8% pellets. Performance and processing metrics were analyzed in a one-way ANOVA using the GLM procedure of SAS. Performance and processing yields were unaffected by PQ during weeks 5-12 ($P > 0.05$), suggesting that PQ may be less important in light hen turkey production. However, PQ dramatically affected hen performance from weeks 13-16 where IPQ feed improved FCR by 0.237 compared to PPQ feed ($P = 0.0126$). Furthermore, weeks 5-16 FCR was improved by 0.107

when IPQ feed was provided ($P=0.0374$). Therefore, producers of heavy hens should consider PQ improvements to elicit performance benefits. Week 16 processing yields were not affected by PQ ($P>0.05$). These data indicate that PQ is an important parameter to consider during the latter phases of hen turkey production when feed intake is highest.

Perhaps feed mill managers and integrators can implement manufacturing techniques that are known to improve PQ later in hen turkey production when PQ is generally poor.

Key Words: pellet quality, turkeys, feed manufacture, processing

Metabolism and Nutrition: Vitamins and Minerals

223 Combined effects of dietary cadmium, lead, mercury, and chromium on performance, egg quality, serum biochemical markers, and oxidative stress in laying hens, and the subsequent attenuation of toxicity with selenized yeast. Cai M. Wu*¹, lang Li¹, Todd Applegate², Shiping Bai¹, Guangmang Liu¹, Keying Zhang¹; ¹*Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu, China,* ²*Poultry Science, University of Georgia, Athens, Georgia, United States.*

Cadmium, lead, mercury, and chromium are frequently found together in the environment and can occur in high concentrations in feedstuffs. The objective of this trial was to investigate the combined effects of dietary cadmium, lead, mercury, and chromium on laying performance, egg quality, serum biochemical markers, and oxidative stress of laying hens, as well as the alleviation of toxicity by dietary supplementation with selenized yeast. A total of 160 Lohmann pink-shell laying hens (63-wk-old) were randomly divided into 4 treatments, each with 10 replicates of 4 hens. The treatments were as follows: control group: corn-soybean meal basal diet; Se group: the basal diet supplemented with 0.4 mg/kg Se from selenized yeast; combined heavy metals group (HEM): the basal diet supplemented with 5 mg/kg Cd(CdCl₂), 50mg/kg Pb (Pb(NO₃)₂), 3mg/kg Hg (HgCl₂) and 5 mg/kg Cr (CrCl₃); Se+HEM group: the HEM diet supplemented with 0.4 mg/kg selenium from selenized yeast. Laying hens were given feed and water *ad libitum*. The experimental period was 12 weeks. The PROC MIXED procedure of SAS (version 9.4, SAS Inst. Inc., Cary, NC, USA) was used to analyze the data. Values of $P < 0.05$ were considered statistically significant, with the trends toward significance indicated by $0.05 < P < 0.10$. Dietary HEM exposure significantly decreased ($P < 0.05$) hen-day egg production and average egg weight, and egg white quality. HEM significantly increased ($P < 0.05$) glutamic oxalacetic transaminase (AST) activity in the serum, induced higher malondialdehyde (MDA) and reactive oxygen species (ROS) in the serum, liver and ovary, and significantly decreased ($P < 0.05$) the activity of superoxide dismutase (SOD) and tended to decrease glutathione S-transferase (GST) ($P = 0.09$) in the serum. Meanwhile, HEM significantly decreased ($P < 0.05$) the activity of SOD, GST, glutathione peroxidase (GPX) and glutathione (GSH) in the liver, and the activity of GPX and GSH in the ovary. Selenium addition of 0.4 mg/kg significantly ($P < 0.05$) increased AST concentration in the serum and SOD activity and tended to increase GST activity ($P = 0.09$) in the serum. Selenium significantly improved ($P < 0.05$) GPX activity in the liver and GPX activity and GSH concentration in the ovary. Therefore, dietary HEM exposure depressed laying performance and egg white quality was likely due to an impaired antioxidant capacity, disrupted hepatic function, and elevated HEM accumulation in egg yolk and egg white of laying hens. Selenium addition of 0.4 mg/kg attenuated

toxicity of HEM on oxidative stress and hepatic function but did not ameliorate toxic effects of HEM on laying performance and egg white quality.

Key Words: laying hens, heavy metals, intoxication, selenized yeast, alleviation

224 No presentation materials submitted.

225 Copper(I) oxide as an alternative for antibiotic replacement to promote growth and improve health in broilers under challenge. Alessandra Monteiro*¹, Denise Cardoso¹, Cristina Massoco Salles Gomes³, Cristiane S. Araujo², Fabricia d. Roque², Lucio F. Araujo²; ¹*Animine, Annecy, Rhone-Alpes, France,* ²*Nutrition and Animal Science, University of Sao Paulo, Pirassununga, Brazil,* ³*Department of Pathology, University of São Paulo, Pirassununga, São Paulo, Brazil.*

The aim of this study was to evaluate the effect of copper(I)oxide (Cu₂O, CoRouge[®], Animine, France) compared to an antibiotic growth promoter on performance and immunity of broilers fed a corn-soybean meal-based diet. Four diets were fed to 600 male broilers (Cobb 500) from 1 to 42 d as follows:(1) negative control (NC): diet without antibiotics and with Cu at requirement level (14.5 mg/kg); (2) positive control (AntB): NC + 50 mg/kg of zinc bacitracin; (3) copper supplemented (CR): NC + 150 mg/kg of Cu from Cu₂O; (4) copper and antibiotic supplemented (AntB+CR). Animals were randomly assigned in one of the treatments and placed in pens of 15 chicks (10 replicates/treatment). At day 7, broilers were subjected to an *Eimeria* challenge by oral gavage with 15x the manufacture recommended dose of Bio-Cocciwet – R live vaccine (Biovet[®]). At day 21, blood was collected from one bird per pen for immunity assessment. Data were analyzed by ANOVA using the GLM procedure of SAS. At 21 days, broilers fed NC had worst ($P < 0.01$) BW and FCR (806g and 1.31, respectively) than the other treatments. The combination AntB+CR had a higher ($P < 0.01$) BW (1087g) than AntB alone (986g); CR treatment had intermediate BW value (1016g). The FCR did not change for AntB, CR and AntB+CR treatments (average of 1.19; $P > 0.01$). At the end of the trial (d42), broilers fed CR or AntB+CR diets had better BW (3079 and 3140g, respectively; $P < 0.05$) and better FCR (1.59 and 1.58, respectively; $P < 0.01$) than broilers fed AntB (2986g and 1.65) and NC (2899g and 1.71). Regarding immunological responses, the CD4/CD8 rate was the same among treatments ($P > 0.01$). However, broilers fed CR had greater ($P < 0.01$) phagocytosis rate (75.4%) than these fed the other treatments (average of 54.9%), indicating a higher capacity to fight against infections and to kill pathogens, and which may explain the better performance. To conclude, Cu₂O showed as good performance as growth promoter antibiotics under mild challenge conditions, and providing additional benefits in immunity.

Key Words: Antibiotic, Broilers, Copper, Eimeria

226 **Withdrawn.**

227 Determination of the standardized digestible calcium requirement of broilers from day 11 to 24 post-hatch. Carrie L. Walk^{*1}, Zhenzhen Wang², Shikui Wang², Jose O. Sorbara³, Jingcheng Zhang²; ¹DSM Nutritional Products, Heanor, United Kingdom, ²DSM Nutritional Products, Bazhou, China, ³DSM Nutritional Products, Kaiseraugst, Switzerland.

An experiment was conducted to determine the standardized ileal digestible (SID) calcium requirement of Arbor Acres Plus male broilers from d11 to 24. Broilers (n = 576) were obtained at hatch and allocated to 96 cages with 6 birds/cage. From hatch to d24, 16 randomly allocated cages of birds were fed a nutrient adequate positive control (PC) diet. The PC was formulated to contain 0.96 and 0.87% total Ca from hatch to d10 and d11 to 24, respectively. The remaining 80 pens were fed a nutrient adequate diet, formulated to contain 0.50% SID Ca (1.03% total Ca) and 0.48% available P (avP) from hatch to d10. On d11, five diets, containing 0.56, 0.46, 0.36, 0.26 or 0.16% SID Ca were randomly assigned to 16 cages/diet. Available P was maintained at 0.48% from hatch to d10 and 0.44% from d11 to 24, including 0.19% avP from 3,000 FYT/kg of phytase in the SID Ca diets. The same ingredients were used in all experimental diets. Data were analyzed in JMP Pro v. 15.1. Means were separated using orthogonal contrasts to compare the PC vs all SID Ca diets and linear and quadratic effects of SID Ca. From hatch to d10, broilers fed diets formulated to contain 0.50% SID Ca tended to eat more (P = 0.07), gained more (P = 0.001), and tended to be more (P = 0.06) efficient than birds fed the PC. From d11 to 24, there was no effect of diet (PC vs SID Ca) or graded levels of SID Ca on FI or BWG. Birds fed the PC were more efficient (P = 0.02) than birds fed graded concentrations of SID Ca; most likely due to the quadratic (P = 0.04) effect of SID Ca level on feed conversion ratio, which was highest in birds fed 0.46 or 0.16% SID Ca and lowest in birds fed 0.26% SID Ca. Tibia ash percent was greatest in birds fed 0.56 or 0.46% SID Ca and decreased (quadratic, P = 0.04) as the SID Ca content in the diet decreased. This resulted in a lower tibia ash percent (P < 0.001) in birds fed the SID Ca diets compared with birds fed the PC. Apparent ileal digestibility (AID) and apparent retention of Ca or P were greater (P < 0.0001) in birds fed the diets formulated using SID Ca compared with birds fed the PC. This was likely due to the greater AID of Ca in birds fed 0.16 or 0.26% SID Ca compared with birds fed 0.56 or 0.46% SID Ca (quadratic, P = 0.01). The AID of P or apparent retention of Ca increased (linear, P < 0.0001) as the dietary SID Ca concentration decreased from 0.56 to 0.16%. Apparent P retention was greatest in birds fed 0.36% SID Ca (quadratic, P < 0.0001) and decreased as dietary SID Ca decreased. Regression equations developed using apparent P retention or percent tibia ash suggested the SID Ca requirement of 11 to 24 d-old broilers was 0.42 or 0.50%, respectively. This

corresponds to a SID Ca:avP ratio of 0.95 to 1.14.

Key Words: digestible calcium, phosphorus, phosphorus digestibility, phytate-free, tibia ash

228 Available phosphorus and calcium: are we close to the best dietary levels for performance and mineralization in broilers regarding calcium to available phosphorus ratio? Marion Bournazel^{*}, Stéphanie KLEIN, Pierre Moquet, Adeline Mathiaud; R&D, Mixscience, Bruz, France.

Low phosphorus (P) diets may be a key for a sustainable broiler production. However, in fast growing broilers, it is observed that low P diets leads to lower bone mineralization and welfare issues. This may be due to the supply of dietary available P (aP) and of calcium (Ca), and to differences in their utilization. Currently, aP and Ca:aP requirements of modern broilers remain unclear. Consequently, a trial was performed to study the effect of dietary aP and Ca:aP ratio on performance of broilers. A total of 384 day old male Ross 308 broilers were randomly assigned to 6 groups, each replicated 16 times, for 42 days (d). There were 3 growing phases, with starter (d0-d10), grower (d11- d21) and finisher (d22 -d42). Groups consisted in different feeding with 3 sequences with low (L), medium (M) and high (H) aP levels in starter (L:0.45; M:0.50, H:0.55), grower (L:0.35, M:0.40, H:0.45) and finisher feeds (L:0.27, M:0.32, H:0.37) with two Ca:aP ratio (low: 1.8 or high: 2.2). Groups were arranged as follows: LLL2.2, MMM2.2, HHH2.2, HHL2.2, LLL1.8 and HHH1.8. Orthogonal contrasts were performed for statistical analysis to either detect the effect of Ca:aP ratio, the linear or quadratic effect of aP, or the effect of the decrease of aP in finisher phase. Dietary treatments did not affect performance during the starter and grower phase. In the finisher phase, average daily gain and feed conversion ratio (FCR) tended to improve linearly with increasing aP level at the highest Ca:P ratio (P<0.1). A decrease of performance was observed with low aP in finisher phase (1.78 FCR for HHH2.2 vs 1.87 for HHL2.2), suggesting a high aP requirement during this phase. Concerning bone characteristics, no effect was observed at 21 d. At 42 d, the decrease of aP in HHL2.2 led to lower tibial breaking strength (TBS) and stiffness (TS), resulting in values similar to LLL2.2. In contrast, HHH2.2 resulted in a higher tibial TBS and TS compared to HHL2.2 and LLL2.2 (P<0.05). LLL1.8 resulted in the lowest tibial TBS and TS values of all groups. HHH1.8 did not differ from HHH2.2 for those parameters. Concerning carcass traits, LLL2.2 had a significantly higher breast meat weight (+65 g; P<0.05) and yield than LLL1.8 (+1.1 pt; P<0.05). In conclusion, high aP with high Ca seemed to be the best strategy for the studied criteria. At low Ca:aP ratio, low aP was the worst strategy. Conversely, high aP led to the highest bone strength, irrespective of Ca supply. The decrease of aP supply degraded growth in finisher phase and bone characteristics at d42, and therefore does not seem to be a beneficial strategy. Future trials are needed to investigate the effect of reducing aP in starter and grower

phases, e.g. with LLH2.2 or LLH1.8 sequences.

Key Words: available phosphorus, calcium, Ca:aP ratio, mineralization, broiler

229 Evaluating the impact of supplementing organic chromium with flax seed in broiler diets: Effects on production performance, breast muscle pathology, and meat quality aspects. Ahmad Fraz*^{GS 1}, Sydney T. Bolanos¹, Nathan B. Parker¹, Christiane V. Loehr³, Gita Cherian²; ¹*Animal and Rangeland Sciences, Oregon State University, Corvallis, Oregon, United States*, ²*Animal and Rangeland Sciences, Oregon State University, Corvallis, Oregon, United States*, ³*Department of Biomedical Sciences, Carlson College of Veterinary Medicine, Oregon State University, Corvallis, Oregon, United States*.

The study investigated the impact of organic chromium (Cr) and flax supplementation on live performance, carcass yield, serum metabolites, muscle lipid profile, histopathological aspects, and meat quality parameters in broilers. Ninety (n=90), day-old Cobb chicks were fed a corn-soybean meal-based diet containing 0% flax (Control), 10% flax (Diet 1) and Diet 1 + 0.05% organic Cr (Diet 2) for 42 days. The chicks were kept in 6 pens with 5 chicks per replicate pen. Body weight (BW) gain and feed:gain was determined at days (1-11), (11-22), and (22-42). For all response variables, the effect among dietary treatments was compared separately with one or two-way ANOVA using SAS 9.4. P-values were considered significant at ≤ 0.05 . On day 22, Diet 1 and Diet 2 chicks had lower BW gain and feed:gain than Control ($P < 0.05$). In the finisher phase (22-42 days), Diet 2 chicks were higher in BW with improved feed:gain than Diet 1 ($P < 0.05$) but were not different from Control chicks ($P > 0.05$). The overall weight gain and feed:gain was higher in Diet 2 and Control than Diet 1 ($P < 0.05$). The relative organ weight (liver, fat pad, heart, gizzard) and yield of breast and thigh muscles were not different ($P > 0.05$) among the diets. No difference was observed in the visual incidence of white striping score (1= no striping, 2= mild, 3= severe) on breast muscle among diets ($P > 0.05$). Histopathological changes including floccular/vacuolar degeneration, fibrosis, lipidosis, interstitial inflammation, and muscle lysis were less pronounced in Diet 1 than Diet 2 ($P < 0.05$). Breast muscle total fat and cholesterol were lower in Diet 1 than Control ($P < 0.05$). Serum cholesterol and aspartate aminotransferase were lower in Diet 1 and Diet 2 than in Control ($P < 0.05$). Total serum proteins were lower in Diet 2 than other diets ($P < 0.05$). Diet 1 and Diet 2 had increased (>2 -5 fold) total and long-chain ($>20C$) n-3 fatty acids (FA) in the breast and thigh muscle ($P < 0.05$). Total saturated FA was decreased in the thigh muscle of flax-fed birds compared to Control ($P < 0.05$). In contrast, total monounsaturated FAs were highest in the breast and thigh muscle of Diet 2 ($P < 0.05$). Lipid peroxidation products measured as thiobarbituric acid reactive substances were lower in the breast muscle and serum of Diet 1 and Diet 2 than Control ($P < 0.05$). No effect

of diet was noticed for breast muscle pH, color, tenderness, and cook loss values ($P > 0.05$). Drip loss values were reduced for Diet 1 and Diet 2 versus Control ($P < 0.05$). As consumer demand for n-3 FA-rich poultry products is on the rise, Cr may serve as a feed supplement that could be used in broilers fed flax-based diets for enriching edible tissues with n-3 FA, while enhancing production performance.

Key Words: broiler, flax, chromium, n-3 fatty acid, meat quality

230 Effects of *in ovo* injection of 25-hydroxyvitamin D₃ in conjunction with Marek's Disease vaccine on the hatchability and hatch variables of Ross 708 broilers. Noelle M. Forcier*^{UG1}, Saman Fatemi¹, Christopher J. Williams², Katie E. Elliott³, Ayoub Mousstaaid¹, David Peebles¹; ¹*Poultry Science, Mississippi State University, Starkville, Mississippi, United States*, ²*Zoetis, Swabia Court, North Carolina, United States*, ³*Poultry Science, Mississippi State University, Starkville, Mississippi, United States*.

The *in ovo* injection of 25-hydroxyvitamin D₃ (25OHD₃), the second metabolite of vitamin D, has been shown to improve the hatchability, live performance, breast meat yield, small intestine morphology, and immunity of broilers. However, effects of the combined *in ovo* injection of different levels of 25OHD₃ and a commercial Marek's Disease vaccine (HVT) on broiler chick hatchability have not been previously investigated. Therefore, the purpose of this study was to determine their combined effects on live embryonated broiler hatching egg hatchability (HI), hatch residue, and hatchling BW at 528 h of incubation (hoi) subsequent to their *in ovo* administration at 432 hoi. A total of 1,800 live embryonated hatching eggs from 35 wk-old commercial Ross 708 broiler breeder hens were assigned to one of the following 5 *in ovo* injection treatments: 1) non-injected (control); or a 50 μ L solution volume of 2) commercial diluent containing 0.6 μ g of 25OHD₃; 3) 25OHD₃-0.6 (commercial diluent containing 0.6 μ g of 25OHD₃); 4) 25OHD₃-1.2 (commercial diluent containing 1.2 μ g of 25OHD₃); and 5) 25OHD₃-2.4 (commercial diluent containing 2.4 μ g of 25OHD₃). A water-soluble form of the 25OHD₃ (Hy-D® 1.25%; DSM Nutritional Products Inc., Parsippany, NJ) was used. All eggs were candled at 312 and 430 hoi, and percentage egg weight loss (PEWL) between 0 and 312, 312 and 430, and 0 and 430 hoi was determined. One egg from each of the 5 treatment groups on each of the 6 incubator tray levels (30 total eggs) was randomly selected for embryo staging analysis. A randomized complete experimental block design was employed and all data were analyzed by one-way ANOVA using SAS (9.4) software. No significant treatment differences were observed for HI or PEWL in any of the time intervals. However, hatchling BW was higher ($P = 0.014$) in the 25OHD₃-1.2 and 25OHD₃-2.4 treatment groups as compared to the sham control and 25OHD₃-0.6 treatment groups. Also, embryo mortality during the pipping process was higher ($P = 0.05$) in the 25OHD₃-2.4 in conjunction with a Marek's Disease

vaccine has a promising result on hatchling BW although the higher level may increase embryonic mortality. Further research is needed to determine Marek's Disease vaccine viability and the post-hatch effects of various levels of 25OHD₃ when both are administered together

Key Words: 25-hydroxycholecalciferol, Broilers, Hatchability, In ovo injection, Marek's Disease vaccine.

231 25-hydroxycholecalciferol reverses heat induced alterations in bone quality in finisher broilers associated with effects on intestinal integrity and inflammation.

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Alterations in ambient temperature have been associated with multiple detrimental effects on broilers such as intestinal barrier disruption and dysbiosis resulting in systemic inflammation. Inflammation and 25-hydroxycholecalciferol (**25-OH-D₃**) have shown to play a negative and positive role, respectively, in the regulation of bone mass. Hence the potential of 25-OH-D₃ in alleviating heat induced bone alterations and its mechanisms was studied. 15-day-old birds were randomly allotted to control (22°C, *ad libitum* feeding basal diets), heat stress (daily 7 h at 34°C and rest of day 26°C, *ad libitum* feeding basal diets), pair feeding (22°C, restrictive feeding according to feed intake of heat stress-birds of basal diets) or 25-OH-D₃ (heat stress, *ad libitum* feeding basal diets supplemented with 0.069 mg/kg 25-OH-D₃) group. Heat stress exposure from 22 to 39 days. Data were analyzed using One-way the analysis of variance (**ANOVA**). Heat stress directly induced a decrease in tibia material properties and bone mass, as demonstrated by lower mineral content, and heat stress caused a notable increase in intestinal permeability. Treatment with dietary 25-OH-D₃ reversed the heat stress-induced bone loss and barrier leak. Broilers suffering from heat stress exhibited dysbiosis and increased expression of inflammatory cytokines in ileum and bone marrow, as well as increased osteoclast number and activity. Changes that were prevented by dietary 25-OH-D₃ administration. Specifically, dietary 25-OH-D₃ addition decreased abundance of B- and T-cells in blood, and the expression of inflammatory cytokines in both ileum and bone marrow, but did not alter the diversity and repopulation of major bacterial phyla. With regard to bone remodeling, dietary 25-

OH-D₃ supplementation was linked to a decrease in serum C-terminal cross-linked telopeptide of type I collagen reflecting bone resorption and a concomitant decrement in osteoclast-specific marker genes expression (e.g. cathepsin K), whereas it did not apparently change serum bone formation markers during heat stress. These data underscore the damage of heat stress to intestinal integrity and bone health, as well as that dietary 25-OH-D₃ supplementation was identified as a potential therapy for preventing these adverse effects.

Key Words: heat stress, intestinal barrier, inflammation, bone remodeling, tibial mass

232 Effects of oil quality, phytase and vitamin E supplementation on the growth performance, nutrient utilization and relative fat and liver weight in 21-day-old broiler chickens. Richard Adefioye*^{GS}, Merlin Lindemann, Anthony Pescatore, Michael Ford, Sunday Adedokun; *Animal Science, University of Kentucky, Lexington, Kentucky, United States.*

The objective of this study was to determine the effect of oil quality, phytase, and vitamin E supplementation on the performance, relative liver and fat weight, and the apparent total tract utilization of nutrients in 21-day-old broiler chickens. The experiment used 378 day-old male by-product Cobb breeder chicks with 9 treatments consisting of 7 replicates of 6 birds per replicate cage. The first 8 treatments were based on a marginally non-phytate phosphorus (nPP) deficient diet (0.31%), while treatment 9 was a positive control (PC) with adequate nPP levels (0.45%). Specifically, the treatments included 2 oil quality levels (fresh soy oil; peroxide value (PV) = 3 meqO₂/kg and oxidized soy oil; PV = 109 meqO₂/kg), 2 phytase levels (0 vs 1000 FTU/kg), 2 levels (0 and 150 ppm) of dietary vitamin E (mixed tocopherols containing 55-75% γ -tocopherols) and the PC. All birds were fed the PC diet from days 0 to 7 before placing them on the experimental diets. The 8 marginal nPP treatments were analyzed using the GLM procedure of SAS appropriate for a factorial arrangement of treatments (2x2x2). The effect of marginal nPP was tested using contrast between the PC and the nPP deficient diet containing fresh oil without phytase or vitamin E supplementation. The PC diet resulted in lower ($P < 0.05$) total tract P utilization but a higher ($P < 0.05$) bone breaking strength (BBS) and ash content compared to the diet with deficient nPP level without phytase or vitamin E supplementation. Phytase supplementation improved ($P < 0.05$) feed efficiency (d 7-14 and 7-21), Ca utilization, BBS, tibia ash, the apparent metabolizable energy (AME), and AME corrected for nitrogen (AMEn) but decreased ($P < 0.05$) bone lipid content. Oxidized oils resulted in a higher ($P < 0.05$) relative liver weight, and AME. Birds that received oxidized oil and phytase had a higher ($P < 0.05$) AME and AMEn compared to birds fed diets containing either fresh or oxidized oil without phytase supplementation. Crude fat utilization was higher ($P < 0.05$) in birds that received oxidized oil without phytase

supplementation, compared to those that received oxidized oil with phytase and those that received fresh oil with phytase. Furthermore, the birds that were fed diets containing oxidized oil with no vitamin E had higher ($P < 0.05$) utilization of crude fat compared to those fed oxidized oil with vitamin E. The results of this study show that phytase supplementation is beneficial for improving feed efficiency, Ca utilization, BBS, tibia ash, AME, and AMEn regardless of oil quality or vitamin E supplementation. Furthermore, when feeding oxidized oils, phytase supplementation improved AME and AMEn but reduced crude fat utilization.

Key Words: oil quality, oxidized oils, vitamin E, phytase, broiler

233 Effects of dietary Ca concentration on the performance of broiler chicks fed various sources of dietary P with and without phytase supplementation, responses under slight P deficiency. Cooper Fritzen*^{GS 1}, Mike Bedford², Mike Persia¹; ¹Virginia Tech, Blacksburg, Virginia, United States, ²AB Vista, Marlborough, United Kingdom.

A follow up experiment was conducted to determine the effects of high dietary calcium (Ca) on broiler chicks fed diets containing moderately low phosphorus (P) from either nonphytate (nPP) or phytate P (PP) with or without phytase. Newly hatched broiler chicks were housed in raised-wire starter batteries (65.8 in²/bird) for 16 d. The 12 treatments were replicated 6 times using 8 chicks per cage resulting in 576 Hubbard x Cobb 500 chicks. The 3 x 2 x 2 factorial arrangement included 3 P treatments (0.325% nPP, 0.325 + 0.1% P from nPP and 0.325 + 0.1% P from PP), 2 concentrations of Ca (0.9 vs. 1.4% Ca) with and without phytase (0 or 1,000 FTU of phytase). Body weight gain (BWG), feed intake, mortality corrected feed conversion ratio was calculated over the 16 d period. On d 16, all remaining chicks were euthanized, and the right tibias were collected and analyzed for tibia ash weight (TAW) expressed as mg/tibia and as a percentage (TAP). All data were analyzed as a 3 x 2 x 2 factorial using ANOVA in JMP 14 ($P \leq 0.05$). There were Ca x phytase interactions for BWG and FI, but not for FCR ($P \leq 0.05$). Although BWG and FI were maximized in the 0.9% Ca fed diets, phytase was able to partially ameliorate the negative effects when 1.4% Ca diets were fed (For BWG 351.0 g vs. 389.8 g and FI 3.94 kg vs. 4.31 kg without vs. with phytase respectively, $P \leq 0.05$). There was a three-way interaction for TAW ($P \leq 0.05$). Phytase increased TAW in all treatments with the exception of the 0.9% Ca and 0.325 + 0.1% P from nPP fed birds where P utilization may have already been maximized. When TAP was considered, 2-way interactions were noted for P x Ca and P x phytase. The increased Ca in the diets had a larger negative effect on the diets without P supplementation and those supplemented with nPP (dicalcium phosphate) in comparison to those supplemented with PP (32.7% and 38.6% vs. 39.4% respectively, $P \leq 0.05$). Both supplemental nPP and PP increased TAP

without phytase, but when these diets were supplemented with phytase, the diet with nPP resulted in a decreased improvement in TAP in comparison to PP (42.3% vs. 43.7% respectively, $P \leq 0.05$). This response is possibly associated with diminishing returns as the nPP supplemented diet approach the birds' nPP requirement. When diets were formulated to contain P concentrations that were more relevant to industry conditions, responses were more difficult to quantify than those observed at lower dietary P concentrations. However, phytase supplementation did partially ameliorate the negative consequences of high Ca. The use of nPP vs. PP is complex, as it appears that supplemental PP is better utilized in high Ca feeds when compared to supplemental nPP.

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

234 Available phosphorus requirement for broilers: integrated assessment of performance results using meta-analysis. Carolina Haubert Franceschi*^{GS 1}, Thais Bastos Stefanello², Alexandre B. Mariani¹, Marcos Kipper³, Ana P. Ázara De Oliveira³, Vinicius Gonsales Schiramm³, Cícero Peres da Cruz³, Ronan O. Fernandes dos Santos³, Ines Andretta¹; ¹Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil, ²Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, ³Poultry Study Group, UFRGS, Porto Alegre, Brazil.

Phosphorus is an essential nutrient that plays an important role in many biological functions. This study was developed to reassess the available phosphorus (aP) requirements of broilers by carrying out a meta-analysis of previously published performance responses. Studies available in electronic databases (PubMed, Web of Science, and Scopus) were searched using systematic review methods. Then, publications were selected for the meta-analysis if presenting performance responses of broilers fed at least three dietary aP levels. Data from 76 studies (64,503 animals from 1 to 53 days old) published from 1997 to 2019 were extracted to an electronic spreadsheet. Due to the high variability among studies, the aP concentration and the performance responses were standardized. First, the aP intake was relativized to the recommendations of Brazilian Tables for Poultry and Pigs (i.e., values were considered 100% if in complete agreement with the recommendations of Rostagno et al., 2017, 4th edition, Viçosa, Brazil). After, the average daily weight gain (ADG) and feed conversion ratio (FCR) were also expressed as relative values to the best response in the study (i.e., best performance in each study was considered 100%). Linear broken-line (LP), quadratic broken-line (QP), and exponential asymptotic (EXP) models were used to estimate the relative aP intake that maximized performance responses (NLMIXED procedure with random effect of study, SAS 9.3). When using LP model, the relative aP intake required to maximize ADG was 71% of the recommendations from Brazilian Tables. The values obtained with QP and EXP models were 85 and

104% of Brazilian Tables recommendations, respectively. The results for FCR were estimated at 57 (LP) and 94% (QP) of Brazilian Tables recommendations. In this last case, EXP model failed to converge. The LP models showed the lowest Akaike Information Criterion for both responses. Interaction of the response of broilers to increasing aP intake was found ($P < 0.05$) for growing phase (ADG and FCR) and for the use of animal origin ingredients (ADG), while was not significant for phytase, feed form or calcium level. Reassessing the aP recommendations is essential for the implementation of precision feeding strategies and the meta-analysis is a suitable tool in this very important task.

Key Words: minerals, phosphorus, systematic review

235 Available phosphorus requirement for broilers: integrated assessment of bone characteristics using meta-analysis. Thais Bastos Stefanello*^{GS 1}, Carolina Haubert Franceschi¹, Alexandre B. Mariani², Marcos Kipper¹, Ana P. Ázara De Oliveira³, Vinicius Gonsales Schiramm³, Cícero Peres da Cruz³, Ronan O. Fernandes dos Santos³, Ines Andretta¹, Andréa M. L. Ribeiro¹; ¹Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, ²Animal Science, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil, ³Poultry Study Group, São Paulo, Brazil.

Precise estimations of available phosphorus (aP) requirements are essential in modern broiler production. Although several studies have been conducted to study aP requirements, no definitive consensus has been achieved regarding aP ideal recommendation. This meta-analysis was conducted using results published in the literature to evaluate the requirements for aP to maximize tibia characteristics in broilers. The research employed three different electronic databases (PubMed, Web of Science,

and Scopus). All publications were critically evaluated. For inclusion in the database, the articles must compare at least three dietary levels of aP. After applying the selection criteria, data from 47 scientific publications (35,592 animals) published from 2000 to 2019 were extracted to an electronic spreadsheet. Standardization procedures were adopted to deal with the high variability among studies. Thus, raw values of tibias ash, phosphorus (P), and shear force were expressed as relative to the greatest response in the study (i.e., greatest value for each variable in each study was considered 100%). Relative aP intake was also evaluated according to its relation to the recommendations of Brazilian Tables for Poultry and Pigs (i.e., values were considered 100% if in complete agreement with the recommendations of Rostagno et al., 2017, 4th edition, Viçosa, Brazil). Linear broken-line (LP), quadratic broken-line (QP), and exponential asymptotic (EXP) models were used to estimate the relative aP intake that maximized bone characteristics (NLMIXED procedure with random effect of study, SAS 9.3). Relative aP intake required to maximize tibia ash, P, and shear force were, respectively, 89, 89, and 88% of the recommendation proposed by the Brazilian Tables when using LP models. The QP and EXP models estimated higher relative aP intake to maximize tibia ash (QP: 111%; EXP: 113%), P content (QP: 137%), and shear force (QP: 104%; EXP: 116%). However, the LP models showed the best (lowest) Akaike Information Criterion for all studied responses. Interaction of the response of broilers to increasing aP intake was found ($P < 0.05$) in the growing phase for all studied responses, and also for phytase and feed form when evaluating tibia ash. No interaction was found for the use of animal origin ingredients or calcium level. Integrating available results to reassess the aP recommendations is an important task toward feeding programs that maximize the efficiency of P utilization in broilers.

Key Words: Ash content, bone quality, shear force, systematic review, tibia

Microbiology and Food Safety

236 Contamination of eggs by *Salmonella* Enteritidis and *Salmonella* Typhimurium in experimentally infected laying hens in indoor cage-free housing. Richard K. Gast*¹, Deana R. Jones¹, Rupa Guraya¹, Kenneth E. Anderson², Darrin M. Karcher³; ¹*US National Poultry Research Center, USDA-ARS, Athens, Georgia, United States*, ²*Poultry Science, North Carolina State University, Raleigh, North Carolina, United States*, ³*Animal Science, Purdue University, West Lafayette, Indiana, United States*.

Contaminated eggs are a leading source of human *Salmonella* infections and this problem continues to challenge public health authorities and egg industries around the world. *Salmonella* invasion of the ovaries and oviducts of infected laying hens can result in bacterial deposition inside the edible portions of developing eggs. The introduction, persistence, and transmission of salmonellae in commercial egg-laying flocks are influenced by flock management practices, but the food safety ramifications of different types of laying hen housing remain unresolved. The present study assessed the frequency of internal contamination of eggs after experimental *Salmonella* Enteritidis and *S.* Typhimurium infection of laying hens in indoor cage-free housing. Groups of 72 hens were housed on wood shavings in isolation rooms simulating commercial cage-free barns with community kick-out nest boxes and perches and 1/3 of the hens in each room were orally inoculated with 8.0×10^7 cfu of 2-strain mixtures of either *S.* Enteritidis (2 rooms) or *S.* Typhimurium (2 rooms), and the entire internal contents of all eggs laid 5-30 d post-inoculation in nest boxes or on the flooring substrate were cultured to detect *Salmonella*. Contaminated eggs were laid between 8 and 28 d post-inoculation. The contamination frequencies associated with the two egg collection locations were not significantly different ($P < 0.05$ in Fisher's exact test). The overall incidence of *S.* Enteritidis isolation from eggs (3.41%) was significantly ($P = 0.0005$) greater than *S.* Typhimurium (1.19%). These results demonstrate that oral infection of a relatively small proportion of laying hens in indoor cage-free housing with invasive *Salmonella* serovars can result in the production of internally contaminated eggs at low frequencies over a period of nearly a month post-inoculation.

Key Words: *Salmonella* Enteritidis, *Salmonella* Typhimurium, laying hens, eggs, cage-free housing

237 Impact of different caging systems on eggshell cuticle quality and *Salmonella* adherence in table eggs. Garima Kulshreshtha*¹, Cristina Benavides Reyes², Alejandro R. Navarro², Ty Diep³, Maxwell T. Hincke^{1, 4}; ¹*Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada*, ²*Departamento de Mineralogía y Petrología, University de Granada, Campus de Fuentenueva, Granada, Spain*, ³*Lyn Egg production and Grading, Burnbrae Farms Limited, Lyn, Ontario,*

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Food safety of table eggs is critical, as consumption of contaminated eggs and egg products continues to be linked to outbreaks of food poisoning. Egg producers in the United States and Canada are increasingly introducing alternative caging systems into their production chain, as animal welfare concerns become of greater relevance to today's consumer. Alternative caging systems are designed to allow birds to express a fuller repertoire of normal behaviours due to opportunities for interaction with their environment and other birds. Hen aggression and nesting behaviour is modified in furnished cages, and thus caging system can alter the physiology of egg formation and cuticle deposition / quality. The completeness of cuticle coverage and its chemical composition play an important role in limiting bacterial entry; however, bacterial load on the eggshell surface is another key factor. The goal of this study is to identify the impact of caging system on eggshell cuticle quality and structure of cuticle pore plugs; and to evaluate the role of cuticle on bacterial load. Cuticle pore plugs (n=18) were visualized using scanning electron microscopy and pore plug elemental composition was determined by Energy Dispersive X-Ray Spectroscopy. The inner surface of the pore plug was consistently found to be chemically different from the surrounding eggshell mineral. Cuticle quality and its chemical composition (n=30) was determined by attenuated total reflection Fourier transform infrared spectrometry (ATR-FTIR). Total cuticle was higher in free range and enriched cages as compared to free run, while carbonates and sulphates were higher in free run than enriched cages. Cuticle protein parameters including OH (R=-0.9560) and total cuticle (R=-0.9681) were negatively correlated with the mineral carbonate signal. Bacterial cell attachment assay (n=18) showed that numbers of adhering *Salmonella* Typhimurium on outer surface of eggshells was significantly higher in bleach treated control eggs without cuticle as compared to eggshells from different housing systems (P=0.0001). Significant reduction (P=0.025) in bacterial cell counts was observed in eggs obtained from free range as compared to enriched systems. These results indicate that nesting environment and behaviour of birds can impact bacterial adherence and cuticle parameters. The outcome of our research will provide new tools for predictive microbiology in order to improve the food safety of table eggs.

Key Words: eggshell, cuticle, caging systems, antimicrobial, pathogens

238 Tissue colonization and egg and environmental contamination associated with experimental infection of laying hens with *Salmonella* Braenderup. Javier S. Garcia*, Richard K. Gast, Deana R. Jones; *US National Poultry Research Center, Egg Safety and Quality Research*

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Salmonella is a major foodborne pathogen of concern with the focus on Salmonella Enteritidis for the egg industry. In recent years, other serovars of Salmonella, such as Salmonella Braenderup, have been linked to foodborne illness outbreaks associated with shell eggs. However, little is known of Salmonella Braenderup infections in laying hens. The objective of this study was to investigate the impact of Salmonella Braenderup on organ invasion, as well as egg and environmental contamination. Cage-free specific-pathogen-free laying hens (Trial 1 and 2, 43 – 45 weeks old; Trial 3 and 4, 54 – 56 weeks old; 72 hen/trial) were orally challenged with 7 log cfu of the egg-associated human outbreak isolate of Salmonella Braenderup (SB). On day 6 post-inoculation, half of the challenged flocks (36 hens/trial) were euthanized and tissue samples (ileocecal junction, liver, spleen, ovary, and oviduct) were collected. In trials 1 and 2, eggs were collected from day 7 to day 20 post-inoculation (excluding weekends; 10 days total egg collection/trial) and day 7 to day 27 post-inoculation (excluding weekends; 15 days total egg collection/trial) in trials 3 and 4. Nest box swabs and substrate composite samples were also collected at the time of daily egg collection. Recovery of SB for all samples was analyzed by Chi-square. The rate of SB recovery from organ tissue samples varied between the four trials. In trial 2, 33.3% ($P < 0.05$) of the ileocecal samples were positive for SB, the highest recovery rate of all the trials. Rate of SB recovery from the other organ tissues samples was between 0 and 5.6%. Recovery of SB from egg and environmental samples also varied between the trials. In trial 2, SB was detected in 2.9% of shell emulsions ($P < 0.0043$) compared to the other trials with no detected SB. Salmonella Braenderup was only detected in nest box swabs in trial 2 (7.5%; $P < 0.02$). In trial 1, SB was detected in 92.5% ($P < 0.0001$) of substrate composite samples compared to the other trials (2.5 – 17.5%). In the current study, Salmonella Braenderup was found to invade the organs of laying hens and contaminate eggs at a low rate. Further research is needed to determine how the presence of Salmonella Braenderup in the environment could contaminate shell eggs internally and externally.

Key Words: Salmonella, shell eggs, food safety, layer, environment

239 Efficacy of a combination of a yeast cell wall and live Salmonella vaccine to reduce Salmonella Infantis colonization in broilers to hot rehang carcass rinse. Charles L. Hofacre^{*1}, Sangita Jalukar², Matthew K. Jones¹, Jennie Baxter¹, Roy Berghaus³; ¹Southern Poultry Research Group, Inc., Watkinsville, Georgia, United States, ²Arm and Hammer Animal and Food Production, Mason City, Iowa, United States, ³College of Vet. Med., Animal Science, The University of Georgia, Athens, Georgia, United States.

Salmonella Infantis (S.I.) has been associated with several Poult. Sci. 100 (E-Suppl 1)

foodborne illnesses in the U.S. and around the world. S.I. is a serogroup C1 which the Salmonella vaccines in the U.S. have not been as successful in controlling alone as has been demonstrated for *S. enteritidis* or *S. heidelberg*. The fimbriae of the yeast cell wall has been shown to effectively bind Salmonella in the intestine. Therefore, a study was designed to evaluate the effectiveness of combining a commercially available yeast cell wall product, Celmanax, and the live vaccine MeganVac1. The four treatments with ten replicates were untreated control, live vaccine only, live vaccine plus yeast cell wall (YCW), and yeast cell wall only. The live vaccine was one dose sprayed at day 1 and YCW was at 100 g/metric ton through all feeds to 43 days. On day 4, liver/spleen and ceca were taken from one bird/pen for vaccine take. Then on day 4, 25 of the 50 chicks/pen were challenged with 2.3×10^7 CFU/chick *S. Infantis* by gavage and challenged birds were tagged. The samples collected were 43 day: bootsocks, whole bird carcass rinse (hot rehang) and ceca from five direct and five horizontal challenged per pen. A typical eight hour feed withdrawal was performed prior to catch and processing. Salmonella prevalence and enumeration were performed with tetrathionate, then XLT-4. Enumeration was by micro most probable number (MPN) method of Berghaus et al., 2013. There was no significant impact of the YCW on the live vaccines ability to colonize on day 4. There was no significant reduction in prevalence or number of S.I. in the environmental sample (bootsocks) on day 43 by any treatments. The combined vaccine plus YCW ($P < 0.05$) lowered prevalence of S.I. on carcass rinses (39%^a) versus challenge control (72%^{ab}). The combined vaccine plus YCW also lowered S.I. prevalence in ceca (42%^a) versus control (75%^{ab}). There were numerical reductions in number (MPN/ml) of carcass rinse and MPN/g of ceca by the combined live vaccine plus YCW. In conclusion, the combination of the live *S. typhimurium* vaccine (MeganVac) plus the YCW (Celmanax) demonstrated a consistent reduction in *S. Infantis* prevalence and number in the ceca and in the hot rehang carcass rinse of broilers challenged with the group C *Salmonella Infantis*.

Key Words: Salmonella infantis, live vaccine, yeast cell wall

240 Reducing Salmonella Kentucky in broilers with inactivated autogenous vaccines administered by coarse spray. Charles L. Hofacre^{*1}, Roy Berghaus², Bereket Zekarias³, Sam Christenberry³, Hector Cervantes³, Luis Gomez³, Peter Winter³, Matthew K. Jones¹, Jennie Baxter¹; ¹Southern Poultry Research Group, Inc., Watkinsville, Georgia, United States, ²Poultry Science, The University of Georgia, Athens, Georgia, United States, ³Phibro Animal Health, Teaneck, New Jersey, United States.

Salmonella Kentucky has not often been associated with human foodborne illness. However, it is commonly isolated in carcass and parts rinses in processing plants counting as a positive in a processing plant's FSIS 52-week rolling window. One of the major interventions for broilers has

been one of the two commercially available live *Salmonella* vaccines. However, these vaccines do not afford much reduction in colonization of *S. Kentucky*. In two studies, an autogenous vaccine made from the SPRG challenge *S. Kentucky* investigated the effectiveness of a polymer adjuvant, Carbigen®, delivered by spray. Study 1 design was two replicates (42 chicks/replicate placed) of non-vaccinated and two replicates of autogenous at 0.25 ml/chick coarse sprayed on day 1 and 14. Study 2 design was four replicates (32 chicks/replicate) of non-vaccinated control, autogenous with original adjuvant from study 1 and autogenous with EASE® Technology at 0.25 ml/chick coarse sprayed on day 1 and 14 of age. *S. Kentucky* challenge for both studies (nalidixic acid resistant) was orally gavaged to all birds on day 30 (study 1) and day 29 (study 2) at 0.5 ml/chick (3.0 x 10⁷ CFU/chick, study 1; 7.0 x 10⁷ CFU/chick, study 2). Ceca were aseptically collected from 20 birds per replicate (study 1) or 15 birds per replicate (study 2) and cultured for *Salmonella* prevalence and number by most probable number (MPN) on day 36 and 42/43. Spleen and ½ liver were pooled and cultured for prevalence only on day 36. Both prevalence and MPN per gram of ceca were performed with tetrathionate, then XLT-4 (25 µg/ml nalidixic acid) with the enumeration by micro MPN method of Berghaus et al. 2013. Study 1 had a significant ($P \leq 0.01$) reduction in positive liver/spleen in vaccinated (55%) versus challenge control (80%). There was no significant difference in the ceca prevalence or number on either day 36 or 43. However, the vaccinates had a numerical reduction (MPN/g) in ceca from day 30 to 43. In study 2, the EASE® vaccinates had a 6.7% reduction in liver/spleen prevalence but this was not significant. The S.K. prevalence in the ceca 7 or 14 days post challenge were not significantly reduced. However, both vaccine adjuvant types had significant ($P \leq 0.5$) reduction in S.K. number by MPN/g in the ceca 14 days post challenge. In conclusion, the results of these two studies indicate a spray of the autogenous vaccine with Carbigen® adjuvant reduce the invasion of S.K. into internal organs of liver/spleen. Also, by day 14 post challenge, the vaccinated broilers had less S.K. in their ceca which may impact the number of S.K. that could contaminate the feathers and skin resulting in less *S. Kentucky* entering the processing plant.

Key Words: *Salmonella Kentucky*, spray inactivated vaccine, broilers

241 Effect of a live attenuated *Salmonella Typhimurium*-vaccine against emerging *Salmonella Reading* in poult. Claire Peichel^{*1}, Shijinaraj Manjankattil², Grace Dewi³, Timothy Johnson¹, Sally L. Noll⁴, Anup Kollanoor Johny⁵; ¹*Animal Science, University of Minnesota, St. Paul, Minnesota, United States*, ²*Animal Science, University of Minnesota, Saint Paul, Minnesota, United States*, ³*University of Minnesota, St. Paul, Minnesota, United States*, ⁴*Animal Science, University of Minnesota, Saint Paul, Minnesota, United States*, ⁵*Animal Science, University of Minnesota, St. Paul, Minnesota, United States*.

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Turkey products have recently been implicated in large *Salmonella Reading*-associated outbreaks in the United States and Canada. Because *S. Reading* was previously recognized as a relatively uncommon turkey-colonizing serovar in turkey production, investigating ways to mitigate this understudied pathogen is critical. This study investigated the potential protective effects of Avipro Megan Egg, a live attenuated *S. Typhimurium* vaccine on *Salmonella Reading* in turkey poults in three trials. In the first trial, 24, day-old poults were distributed into one of the three groups: negative control (NC), positive control (PC), or vaccine (VAC). Upon arrival, poults in the VAC group were vaccinated. After 24 hours, poults in the PC and VAC groups were inoculated with a six-strain cocktail of *S. Reading* (~ 4.5 log₁₀ CFU/bird). Twenty-four hours post-inoculation (PI), birds were euthanized, and ceca, liver, and cloaca were collected for *S. Reading* enumeration and enrichment. In the second trial, 24, day-old poults were allocated into groups described above. Birds in VAC were vaccinated upon arrival. After seven days, poults in the PC and VAC groups were inoculated with a six-strain cocktail of *S. Reading* as above. Birds were euthanized at seven days PI, and ceca, cloaca, and liver were collected for *Salmonella* enumeration and enrichment. In the third study, 14, day-old poults were allocated into PC and VAC groups. The VAC group poults were vaccinated on the first day, and all birds were inoculated with 4 log₁₀ CFU *S. Reading* on day 3. On day 4 PI, birds were euthanized, and ceca, cloaca, and liver were collected for *Salmonella* enumeration and enrichment. Ceca were serially diluted, and appropriate dilutions were surface plated on Xylose Lysine Deoxycholate agar (XLD) plates, incubated at 37°C for 24 hours, and *S. Reading* counts were determined. Ceca, cloaca, and liver samples were enriched in selenite cysteine broth and streaked on XLD plates for *Salmonella*. Data were analyzed using the PROC-ANOVA procedure, and significance was tested at $P < 0.05$. In the first trial, the vaccine reduced *S. Reading* by 1.8 log₁₀ CFU/g, compared to the PC ($P < 0.05$). In the second trial, no significant difference was shown between PC and VAC. The third trial resulted in a 0.8 log₁₀ CFU/g (non-significant) reduction of *S. Reading* in the VAC group ($P > 0.05$). There were no significant differences between the samples positive between PC and VAC groups in the three trials, except the first trial in which the VAC group had 50% fewer liver samples positive for *S. Reading*. Results indicate the necessity of a booster dose for *S. Reading* protection and warrant studies in adult turkeys. (NIFA #2020-67017-30787; RARF#81824)

Key Words: *Salmonella Reading*, Vaccination, Poults, Emerging Pathogens

242 Effects of *Eimeria tenella* on cecal luminal and mucosal microbiota in broiler chickens. Philip M. Campos^{*2, 1, 3}, Kate Miska¹, Stanislaw Kahl¹, Jonathan Shao³, Monika Proszkowiec-Weglarz¹; ¹*Beltsville Agricultural Research Center, Animal Biosciences and Biotechnology Laboratory, USDA-ARS, Beltsville,*

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The intestinal disease coccidiosis, caused by parasitic *Eimeria* species, severely impacts poultry production, leading to excessive economic losses. This study aimed to investigate the effects of *Eimeria tenella* (ET) infection on luminal (L) and mucosal (M) cecal (Ce) microbial population. Ross 708 broilers (288) were inoculated with 10^4 ET oocysts (IF) or sham-inoculated (C) at 21 d post-hatch. To investigate CeL and CeM bacterial communities, cecal contents and epithelial scrapings were collected 0, 3, 5, 7, 10, and 14 d post-infection (PI) from C and IF birds for bacterial DNA isolation. At each time point, body weight (BW) and feed intake (FI) were recorded, and body weight gain (BWG) and feed conversion ratio (FCR) were calculated and analyzed using Statistical Analysis System (SAS) software v9.4. The microbiota was determined by sequencing of the hypervariable V3-V4 region of bacterial 16S rRNA (Illumina). Alpha and beta diversity were analyzed using Kruskal-Wallis and PERMANOVA, respectively, on the QIIME 2 2020.11 platform. Differential abundance of bacterial taxa was analyzed using Linear Discriminant Analysis Effect Size (LEfSe). Metagenomic functions were predicted using PICRUSt2 v2.3.0-b. IF birds had significantly lower BWG and higher FCR 7 d PI ($P < 0.05$). In CeL microbiota, observed features (ASVs) and richness (Faith's Phylogenetic Diversity) were lower ($P < 0.05$) 7 and 14 d PI, while evenness was lower 5 d PI, but became higher on 14 d PI ($P < 0.05$) in IF birds in comparison to C birds. Beta diversity analysis showed significant differences between IF and C microbiota 7 d PI (Unweighted UniFrac, $P < 0.05$). In CeM microbiota, Shannon diversity, ASVs, and evenness were lower ($P < 0.05$) in IF birds 7 d PI. Microbiota of IF and C birds significantly differed on 7 d PI (Unweighted UniFrac, $P < 0.05$). LEfSe analysis indicated a higher relative abundance of Enterobacteriaceae (identified as *Escherichia coli* or *Escherichia-Shigella*) in IF compared to C in both CeL and CeM datasets, and substantial increases in Enterobacteriaceae were observed in IF birds 7 d PI. In addition, predicted metagenomic functions related to amino acid biosynthesis were differentially expressed in IF birds, with some of these pathways associated with *E. coli* strain K-12. In conclusion, our results show that ET infection disturbs mucosal and luminal microbiota balance in chickens. Moreover, luminal microbiota seems to be more susceptible to prolonged imbalance due to IF, while mucosal microbiota appeared to be affected only in the short term.

Key Words: microbiota, coccidia, broiler chickens, cecum, 16S

243 Limited gut microbiota overlap between heavy breeders and their specific progeny. Naama Shterzer, Nir

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Introduction: Published microbiota comparisons show similarity between hens and their progeny, implying the possible vertical transmission of commensal gut bacteria from one generation to the next through the egg. On the other hand, another publication showed that exposure to hens greatly affected chick microbiota composition, implying vertical transmission through the egg is limited. Characterizing vertical transmission through the egg is important to develop methods that enhance the transmission of commensals while inhibiting the transmission of pathogens. Furthermore, identifying bacterial phyla which do not transmit effectively through the egg is important, as these bacteria might be important for the development of a normal healthy microbiota. **Objective:** To identify bacterial strains and phylogenetic groups that may be vertically transmitted through the egg. **Materials and methods:** We collected fertilized eggs from ten broiler breeders and grew their chicks for 14 days. We collected fecal samples from the hens during egg collection week and from the chicks at ages 2, 7, and 14 days. We then performed 16S rDNA sequencing and compared microbial communities. **Results:** Out of 410 bacterial strains identified in hen feces only 25 were identified in the chicks by day 2 of life. Furthermore, out of the 410 strains, 250 did not appear in the chicks at least till day 14 of life. PCoA analysis by Jaccard distances showed a progression in the chick's microbiota composition through life. The same analysis also reveals that the chick's microbiota composition is different from the hen's microbiota composition and that the chick's microbiota displays high variation. Last, chick bacterial community analysis identified high levels of potential pathogens such as members of Proteobacteria. **Conclusions:** The few bacterial strains that we identified as common to both hens and 2 day old chicks are possibly transmitted through the egg and warrant further research. However, our results imply that for many bacterial strain transmission through the egg either does not occur at all or is not an effective means of transmission. While we only measured colonization till day 14, this already represents between half to a third of the life span of broilers. Furthermore, while it is possible that colonization does not occur because the gut is not in a developmental state in which these bacteria are able to colonize, published results showing that exposure to the hen enables chick colonization, imply this is not the case. To conclude, further research is required to determine the impact of the lack of transmission of these gut commensals on commercial broilers, and if artificial exposure can positively impact broilers.

Key Words: gut microbiota, vertical transmission, probiotics

244 Development of monoclonal antibodies against *Clostridium perfringens*. Ying Fu*^{1, 2}, Tahrir Alenezi^{1, 2}, Ayidh Almansour^{1, 2}, Hong Wang², Xiaolun Sun^{2, 1}; ¹Cell and Molecular Biology & Poultry Science, University of Arkansas, Fayetteville, Arkansas, United

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Clostridium perfringens is a foodborne bacterial pathogen causing severe necrotic enteritis in chickens and enteritis in humans. Limited effective options are available to treat *C. perfringens*-induced illness. In this study, we aimed to develop monoclonal antibody (mAb) to prevent and treat *C. perfringens*-induced diseases. BL6 wild type (WT) mice were immunized three times with *C. perfringens* sporulation protein (Cp-spor-super) to generate anti-*C. perfringens* B cells. 14 days after the last booster immunization, plasma B cells in the spleen were collected for RNA isolation. Reverse transcription was used to generate complementary DNA (cDNA) from the RNA template. A set of PCR primers were used to amplify antibody variable region of heavy chain (Vh) and variable region of light kappa chain (Vl) from cDNA. The PCR products of Vh and Vl were further assembled into a single-chain variable fragment (scFv) using PCR. All the scFv sequences were inserted into a phagemid of pComb3xSS through digestion with SfiI and then ligation with T4 enzyme. Phage Display with antigen Cp-spor-super in a plate was performed to select the genes expressing anti-*C. perfringens* mAb. Dot-blot was conducted to detect the efficiency of selected phages strongly binding to Cp-spor-super. The results showed that the PCR sizes of Vl and Vh segments were around 350 to 400bp through gel electrophoresis. After gel purification and overlapping PCR, the resulted scFv PCR was around 800bp. After digestion and ligation, the successful construction of recombinant plasmid (pComb3xSS-scFv) was confirmed by double digestion with the NruI and SacI and gel electrophoresis, which showed two bands of 3318bp and 800bp representing the linearization pComb3xSS and scFv, respectively. The scFv phage were generated after transforming pComb3xSS-scFv into *E. coli* TG1 and co-infected with M13O7 phage. The scFv phage was selected through bio-panning in a plate coating with Cp-spor-super. After 2 cycle of bio-panning, the eluted scFv phage showed strong black dot against Cp-spor-super with Dot-blot assay. In conclusion, we have produced anti-*C. perfringens* mAb. The following-up is to identify and characterize the individual mAb for preventing and treating *C. perfringens*-induced enteritis.

Key Words: Necrotic enteritis, *Clostridium perfringens*, monoclonal antibody, phage display, bio-panning

245 Assessment of mycotoxins contamination and chemical composition of South American corn via Near Infrared Reflectance Spectroscopy (NIRS), year 2020. Denize Tyska*^{1, 2}, Daniel F. Soares¹, Gis le P. da Rosa¹, Eduarda d. Gubiani¹, Magdi l A. Reghelin¹, Juliano K. Vidal¹, Adriano O. Mallmann², Carlos A. Mallmann¹; ¹Department of Preventive Veterinary Medicine, Federal University of Santa Maria, Santa Maria, Brazil, ²Pegasus Science, Santa Maria, Rio Grande do Sul, Brazil.

Being widely cultivated throughout the world, corn plays a unique role in the Industry due to its great use in animal Poult. Sci. 100 (E-Suppl 1)

feed, particularly as a basic ingredient in poultry diets. However, grain quality can be altered by the presence of fungi, which produce mycotoxins under favorable conditions. When ingested, these toxins have a negative impact on animal production. Corn grain quality is also affected by its nutritional composition, which varies according to genotype, growing location, and cultivation and processing conditions. It is thus essential to develop reliable methodological tools to analyze this important ingredient. In this context, the present study employed Near Infrared reflectance spectroscopy (NIRS) to examine South American corn samples throughout 2020 in order to assess mycotoxicological contamination and determine water activity (A_w) and chemical composition. Samples were ground in a sampler miller fitted with a 1 mm sieve, homogenized and then scanned on NIRS equipment. A total of 96,921 analyses were performed in corn from Argentina, Bolivia, Brazil, Colombia and Peru. The Brazilian samples were stratified according to the country's five regions. Concentrations of mycotoxins, A_w , crude protein (CP), ether extract, starch and apparent metabolisable energy in poultry were determined in 8,854 spectra. FBs was the most prevalent toxic metabolite in South American corn, being detected in 91.6% of the samples (mean 1,723 $\mu\text{g}/\text{kg}$); ZEN had the second highest positivity, 15.4% (mean 8 $\mu\text{g}/\text{kg}$), followed by AFB₁ with 15.3% (mean 1.2 $\mu\text{g}/\text{kg}$). FBs also had the highest incidence in Brazilian samples, 92.6% (mean 1,833 $\mu\text{g}/\text{kg}$), followed by AFB₁ with 15.2% (mean 1.1 $\mu\text{g}/\text{kg}$) and ZEN with 14.7% (mean 8 $\mu\text{g}/\text{kg}$). Contamination with at least one mycotoxin was found in 93.4% of the samples. A_w index showed oscillations in relation to the means, but the levels were below the safe limit (0.70), thus indicating that the kernels were well stored. There were variations in the chemical composition of the samples. CP ranged from -10% in Colombia to +12.4% in Bolivia in relation to the mean (7.42%). In Brazil, the Southeast region presented the largest positive variability in relation to the means for all evaluated parameters, followed by the Northeast region (excepting for CP); the South region showed the greatest negative oscillation (excepting for CP). The present findings demonstrate that NIRS suits the needs of the Industry for providing information on multiple parameters in real time. Data on prevalence, mycotoxicological contamination, storage conditions and nutritional quality of corn speed up the process of decision making regarding raw material use.

Key Words: Mycotoxins, variability, NIRS, South America, Brazil

246 Correlation between naturally occurring feed mycotoxins and necrotic enteritis severity in broiler birds. Revathi Shanmugasundaram*; *Toxicology and Mycotoxins Research unit, USDA, Athens, Georgia, United States.*

Poultry feeds are naturally contaminated with aflatoxins, fumonisins, deoxynivalenol, acetyldeoxynivalenol, zearalenone and several other mycotoxins. Mycotoxins

increase gut permeability and can exacerbate the severity of necrotic enteritis in poultry. This study quantified and correlated the feed mycotoxin content to the necrotic enteritis disease score and gut permeability. Six experiments were conducted over a period of nine months. In all six studies, birds were raised in cages and challenged with 5×10^3 *Eimeria maxima* oocysts on d14 and with 1×10^8 CFU *Clostridium perfringens* on d19, 20, and 21. All studies were replicated in 8 pens. At d21, three birds per pen were sacrificed and scored for necrotic enteritis disease severity, and gut permeability was measured by FITC-D gavage assay. All studies had the same feed formulation except for the source of grains. Feed was quantified for aflatoxin B1, B2, and G1, fumonisin B1, B2, B3, deoxynivalenol, acetyldeoxynivalenol and zearalenone content by HPLC. The total aflatoxin content of Diets 1 to 6 was 21.2, 5.2, 3.8, 3.5, 3 and 2.5 ppm. Diet 1 had 16.7 ppm aflatoxin B1, while Diets 3, 5, and 6 had no aflatoxin. Diet 3 had the highest concentration of total fumonisins of 3.5 ppm. Diet 4 had 2.3 ppm aflatoxin B1 and 1.5 ppm total fumonisins. Diets 5 and 6 had 366 and 123 ppb of zearalenone. Birds fed Diet 1 had the highest ($P < 0.05$) Wilcoxon/Kruskal-Wallis necrotic enteritis score means, which was significantly higher than the other experiments. Birds fed Diets 5 and six had the lowest Wilcoxon/Kruskal-Wallis necrotic enteritis score means. Analyzing the data from all six experiments, there was a positive correlation between feed total mycotoxin ($r = 0.48$) and total aflatoxin ($r = 0.48$) content with the necrotic enteritis disease severity. There also was a positive correlation between feed total mycotoxin ($r = 0.67$) and total aflatoxin ($r = 0.67$) content with serum FITC-D content. It can be concluded that naturally occurring feed mycotoxins, particularly aflatoxins, at levels above 21.2 ppm exacerbates necrotic enteritis disease severity by increasing gut permeability.

Key Words: Mycotoxins, Necrotic enteritis, Lesion scoring, ELISA, intestinal histomorphology

247 Poultry compost as an alternate safe feed ingredient in broilers production. Haseeb Anwar*, Imtiaz Mustafa; *Physiology, Government College University, Faisalabad, Punjab, Pakistan.*

Poultry litter is being used as a fertilizer in agriculture lands and as a source of food in poultry and livestock since long. But the use of non-processed litter is a source of land and water contamination with NO_3 which could eventually result in respiratory illnesses, cancer and fetal anomalies. Litter composting is viewed as a viable mean for reducing such toxic effects of litter application on agriculture land. The purpose of current study is the introduction and implementation of concept of processed poultry litter as a safe feed additive in broiler poultry. Compost was prepared from dead birds and poultry litter and its nutritional value was confirmed by proximate and mineral analysis and microbial composition was checked by whole genome sequencing (16S RNA) of the DNA extracted from the compost. The results showed no harmful bacteria or toxic

chemicals in the compost. Two hundred and forty, day old broiler chicks were used in the trial and were randomly divided into five treatment groups ($n=48$) with compost supplementation in the broiler feed with a 0, 2.5, 5, 7.5, and 10 percent ratio. On day 42 the birds were slaughtered for the collection of Blood samples which were analyzed for lipid profile, hematology, serum glucose level, total protein, albumin and oxidative stress biomarkers by spectrophotometric colorimetric assays. All the lab values were statistically tested on one way ANOVA using the SPSS software. All the results were non-significant among groups showing no adverse effects as compared with control. In conclusion, composting of poultry wastes (litter, dead birds) can be an efficient procedure for converting wastes into cheap feed ingredient with minimum environmental pollution and health hazard for the birds. The cost of broiler feed could also be reduced by replacing the feed with poultry litter and compost.

Key Words: Litter, Compost, Broiler, Microbiome, Biohealth markers

248 Response of *hilA* of firmly attached *Salmonella* *Infantis* on chicken skin to the use of Cetylpyridinium Chloride (CPC) as a short duration dip. Dana K. Dittoe*¹, Elena G. Olson¹, Lindsey A. Wythe¹, Lindsey Perry², Steven C. Ricke¹; ¹*Meat Science and Animal Biology Discovery, Animal and Dairy Sciences, University of Wisconsin-Madison, Verona, Wisconsin, United States,* ²*Safe Foods, North Little Rock, Arkansas, United States.*

With the emergence of pESI-like megaplasmid among *Salmonella* *Infantis* strains isolated from poultry meat, the poultry industry has become increasingly aware of the enhanced resistance of *Salmonella* *Infantis* to antimicrobials such as peroxyacetic acid (PAA). Therefore, the industry is tasked with identifying effective strategies to reduce these *S. Infantis* strains and potentially minimize virulence gene expression of any *Salmonella* remaining attached to the poultry carcasses. Previously presented data (PSA 2020) demonstrated that the application of PAA and cetylpyridinium chloride (CPC) as short-duration dips were effective in reducing *Salmonella* Typhimurium and *S. Infantis* firmly attached to poultry skin. In the current study, we specifically focused on virulence gene expression in response to these antimicrobials. As such, the objective of the study was to evaluate the efficacy of CPC on reducing *Salmonella* *Infantis* and the expression of *hilA*, a transcriptional regulator of pathogenicity and invasion, on poultry skin compared to the industry standard PAA. Chicken breast skins (4 x 4 cm; $N = 100$, $n = 10$, $k = 5$, per inocula) were inoculated with *Salmonella* (Typhimurium or *Infantis*) and allowed to adhere for 30 min (4 °C) for a final attachment of 10^8 CFU/gram. Skins were then shaken for 30 s to remove excess fluid, and the remaining bacteria were considered firmly attached. Chicken skins were treated with the following short-duration dips (30 s) in 50 mL: a no inoculation - no treatment control (NINTC), no treatment

control (NTC), tap water (TW), TW + 500 ppm PAA (PAA), or TW + 500 ppm CPC (CPC). The excess fluid was shaken off (30 s), and skins were rinsed in neutralizing Buffered Peptone Water and agitated via stomacher (1 min). Skins were discarded, and the rinsates were spot-plated for *Salmonella*. Reverse transcriptase-qPCR (rt-qPCR) was performed targeting the *hilA* gene of the rinsates of the *S. Typhimurium* and *S. Infantis* inoculated skins. The rt-qPCR cycle thresholds of the target gene, *hilA*, and control gene, 16S, were transformed using the $2^{\Delta\Delta Ct}$ method. Data were analyzed using a one-way ANOVA and polynomial contrasts in R with means being separated by Tukey's HSD ($P \leq 0.05$). There was no main effect of treatment on the expression change of *hilA* ($P > 0.05$). However, using polynomial contrasts, the treatment of skins with CPC reduced the expression change of *hilA* in comparison to the controls (NTC, TW, CPC; $P < 0.05$). There were no differences between NTC, TW, and PAA t when polynomial contrasts were performed ($P > 0.05$). In conclusion, CPC is potentially effective in reducing *S. Infantis* *hilA* expression levels on chicken skin when used as a 30 s short-duration dip.

Key Words: poultry, *hilA*, *Salmonella* *Infantis*, peroxyacetic acid, cetylpyridinium chloride

249 Effects of hot water spray and sub-zero saline chilling on bacterial reduction of broiler carcasses. Anisse Pereira*; *Food Science, Cal Poly San Luis Obispo, Los Osos, California, United States.*

Introduction: Reduction of *Salmonella* load on poultry carcasses is one way to prevent salmonellosis. Salmonellosis is a major cause of bacterial enteric illness in both humans and animals. Each year an estimated 1.4 million cases of salmonellosis occur among humans in the United States. The CDC estimates that *Salmonella* in food causes approximately 1,000 hospitalizations and 380 deaths in the United States each year (CDC 2019). The purpose of this research was to evaluate the effects of subzero saline chilling (SSC) with/without hot water spray (HWS) on broiler carcasses for bacterial reduction. **Material & Methods:** A total of 16 commercial broilers in market sizes (6 weeks old in live weights 2.3 – 3.3 kg) were obtained locally. These birds were electrically stunned for 3 s (40 mA, 60 Hz, 110V), bled for 120 s, scalded for 120 s at 56.7°C, and defeathered in a rotary drum picker for 120 s in the Meat Processing Center at California Polytechnic State University. After manual evisceration and washing, four carcasses were randomly assigned to one of the four chilling treatments: 1) water immersion chilling (0% NaCl/0.5 °C) (WIC), 2) HWS (71 °C for 1 min) and WIC, 3) SSC (4% NaCl/-2.41 °C), and 4) HWS and SSC. Data were analyzed using one-way ANOVA and a completely randomized design. A post-hoc analysis was performed using Duncan's multiple range test to evaluate difference among treatments at $P < 0.05$. **Results:** Broiler carcasses in SSC were chilled faster than those in WIC, regardless of HWS. The best bacterial reduction of mesophilic aerobic

bacteria, *Escherichia coli*, and total coliforms was observed on the carcasses chilled in HWS/SSC over WIC, SSC, and HWS/WIC. No *Salmonella* was detected on the carcasses chilled in SSC and HWS/SSC while *Salmonella* was positive on all carcasses chilled in WIC and HWS/WIC. Almost no Gram-negative genus was detected in HWS/SSC when quantitative microbiota profiles of 16 rRNA gene sequences were evaluated. **Conclusion:** Based on these results, the combination of HWS and SSC significantly improved chilling efficiency and bacterial reduction, especially for Gram-negative bacteria.

Key Words: Sub-zero saline chilling, Hot water spray, *Salmonella*, Chilling efficiency, Bacterial reduction

250 Effects of common litter treatments on *Campylobacter jejuni* prevalence in broilers. Luis R. Munoz*¹, Matthew A. Bailey¹, Kaicie S. Chasteen¹, Aidan A. Talorico², John B. Adkins¹, Cesar Escobar¹, Kenneth Macklin¹; ¹*Poultry Science, Auburn University, Auburn, Alabama, United States*, ²*Poultry Science, Auburn University, Auburn, Alabama, United States.*

Campylobacter jejuni is an important zoonotic foodborne pathogen commonly associated with poultry. In humans, it causes acute gastrointestinal disease due to the consumption of contaminated products. *Campylobacter* is shed through feces from contaminated birds into the litter. In commercial settings, litter is reused for multiple flocks which could spread *Campylobacter* if the bacteria survive. Two grow-outs were conducted to evaluate if reusing *Campylobacter* contaminated litter is indeed a possible way to spread the bacteria and to assess the effect of windrow composting and sodium bisulfate poultry litter treatments on prevalence of *C. jejuni* in the litter and the flock. For each grow-out, 1250 chicks were obtained from a commercial hatchery and 50 chicks were randomly assigned to 25 floor pens. In the first grow-out, five birds per pen were inoculated with $7 \log_{10}$ CFU/mL dose of *C. jejuni* on days 14 and 21. The spread of *Campylobacter* was monitored on a weekly basis after inoculation by collecting litter samples (boot swabs) and ceca samples from five uninoculated birds per pen. For the second grow-out, birds were randomly assigned to one of the following treatments: (-) control (fresh litter), (+) control (used litter with no treatment), sodium bisulfate (100 lb./1000ft²), windrow composting or sodium bisulfate (100 lb./1000ft²) and windrow composting. Samples from litter (boot swabs) and ceca were collected at days 14, 21, 28, 35 and 42 for *C. jejuni* enumeration and prevalence estimation. Samples were spread plated onto Campy Cefex blood agar plates for enumeration and incubated for 24 hours at 42 °C in *Campylobacter* Enrichment Broth (3M) to determine prevalence using the 3M Molecular Detection System. The first flock had 100% of the pens (25) and 84% of the birds (105/125) positive for *C. jejuni* at day 28. In the second flock, bacterial counts for the organism were lower than the detection limit in every treatment on each sampling date. Additionally, all the pens (25) and birds (125/125) sampled

were *Campylobacter* negative at the end of the second trial. The results of these studies suggest that *Campylobacter* may not survive in the litter after a three-week downtime period in between flocks. Future studies will continue to evaluate *Campylobacter* survivability on litter to determine the necessary downtime to stop *Campylobacter* spread to a subsequent flock.

Key Words: *Campylobacter jejuni*, Litter contamination

251 Identification of *Enterococcus cecorum* in broiler breeder waterline biofilms and fecal sample. Nicolas Deslauriers*^{GS 2}, Lila Maduro¹, Martine Boulianne¹; ¹*Clinical Sciences, Faculté de Médecine Vétérinaire,, Université de Montréal, St. Hyacinthe, Quebec, Canada,* ²*Clinical Sciences, University of Montreal, Saint-Hyacinthe, Quebec, Canada.*

Following a drop in hatchability, our goals were to identify a bacterial pathogen as a potential source of contamination in broiler breeder barn environmental and fecal samples. Material and methods: Numerous unhatched eggshells and barn environmental swabs, and fecal samples were collected. Bacteriological tests were performed to detect avian pathogens. Confirmation for *Enterococcus cecorum* (EC) was done by Maldi-TOF. Sequencing and genome assembly: As comparison, a known pathogenic EC strain was selected from our lab collection (CECO0008) and all isolated EC genomes were sequenced with NovaSeq 6000 (Illumina). De novo assembly was done after read trimming and contigs aligned against the complete genome of an EC strain (NCTC12421). Detection of virulence factors: All strains were screened for EC known virulence factors: *asa1* (aggregation substance), *gelE* (gelatinase), *hyl* (hyaluronidase), *esp* (enterococcal surface protein), *cylA* (cytolysin), *efaA* (endocarditis antigen), *ace* (collagen-binding protein). Phylogenetic trees: Protein identification was performed, pangenome analysis and core genome alignment were done. Phylogenetic trees were made using the core genome alignment and the aligned nucleotide sequences of the *sodA* gene found in all EC strains. Two Maximum Likelihood trees (core genome alignment and *sodA* gene) were constructed with a bootstrap method (Bootstrap replications=500) and the Jukes-Cantor model nucleotide substitution. Antibiotic resistance profiling: All EC genomes were screened for antibiotic resistance genes using ABRicate with the NCBI database. Results: No pathogenic bacteria was isolated from unhatched eggs. EC was the only known potential pathogen isolated from the egg room humidifier (CECO0002), waterline biofilm (CECO0001) and fecal samples (CECO0003, CECO0004 and CECO0005). CECO0002 was closely related to CECO0004, while CECO0003 and CECO0005, both from fecal samples from the same floor were closely related. None of our strains showed the presence of previously reported virulence genes, but neither did our control pathogenic strain. All tested EC strains did carry 2 to 7 antimicrobial resistance genes. Conclusions: *Enterococcus cecorum* is a new pathogen causing spondylitis and

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osteomyelitis in chickens. It was initially believed to be a commensal of chicken intestines, until pathogenic strains appeared. The origin and source of these strains, where, and whether they survive in a barn environment, are unknown. This is the first report of *Enterococcus cecorum* isolation from the environment, more specifically the drinking water system. Isolated EC strains were multidrug-resistant, hence could serve as a reservoir for resistance genes to other *Enterococcus* spp.

Key Words: *Enterococcus cecorum*, sequencing, waterline biofilm, antimicrobial resistance, waterline contamination

252 Evaluation of a protocol to study *Salmonella* and *Campylobacter* spp. in ovo transmission via inoculation and incubation of hatching eggs. Caitlin Harris*^{GS 1, 2}, L. N. Bartenfeld Josselson², R. J. Buhr²; ¹*Poultry Science, University of Georgia, Athens, Georgia, United States,* ²*Poultry Microbiological Safety and Processing Research Unit, USDA-ARS, Athens, Georgia, United States.*

Salmonella and *Campylobacter* spp. are important foodborne pathogens, and increased knowledge on egg transmission would be beneficial for intervention strategies. There is documentation that *Salmonella* spp. can be transmitted from hen to egg and offspring, but the data is lacking for *Campylobacter* spp. The objective of this project was to evaluate an inoculation and recovery protocol for hatching eggs using *S. Enteritidis* (SE) as a potential way to study egg transmission for both *Salmonella* and *Campylobacter* spp. Two experiments were performed using 2 SE inoculum levels: 10² (exp 1), 10³ (exp2), and buffered peptone water (BPW, negative control). For both experiments, 156 SPF white Leghorn eggs were divided into 2 treatments, SE or BPW inoculated (n=78). On d0, a hole along the equator of the egg was ground and inoculum was injected into the albumen before incubation. Eggs were incubated at 37.5°C, 54% relative humidity, and turned every hr. Three eggs/treatment were sampled on d0 to confirm inoculation, and 30 eggs/treatment were sampled on d5 and d15 of incubation. Embryos were aseptically removed and sampled separately from egg contents. Direct and enriched plating was performed to determine the recovery and mortality of embryos was recorded. For direct counts, ANOVA was used to determine significance; for prevalence and mortality data, the Kruskal-Wallis method was used to determine significance (p≤0.05). For overall mortality, there were no significant differences (p>0.05) between the SE and BPW treatments for exp 1 (16%) and exp 2 (16% vs. 7%). For exp 1 at d5, SE counts were significantly higher (p<0.0001) for both embryos (2.79 log₁₀/mL) and egg contents (1.97 log₁₀/mL) compared to BPW treatment (0 log₁₀/mL). For d15, there were no significant differences (p=0.06) between the SE (0.32 log₁₀/mL) and BPW (0 log₁₀/mL) treatments for embryos, and no egg contents were positive with direct or enriched plating. For exp 2, there was a significant difference (p<0.0001) at d5 for SE recovery from embryos (3.11 log₁₀/mL) and egg contents (3.16 log₁₀/mL) compared

to eggs inoculated with BPW (0 log₁₀/mL). At d15, there was a significant difference (p=0.0036) in direct counts between SE (1.26 log₁₀/mL) and BPW (0 log₁₀/mL) treatments for egg contents. For embryos, there were no significant differences for direct counts, but recovery from enriched plating was significantly higher (p=0.0079) for SE (21% positive) vs. BPW (0% positive) treatments. Results confirm that *Salmonella* Enteritidis can be recovered from both embryos and egg contents of hatching eggs incubated up to 15d and be a potential protocol to study egg transmission. Further experiments with evaluate this protocol with *Campylobacter* spp. as well.

Key Words: Salmonella, Campylobacter, egg transmission, incubation, inoculation

253 Investigation of the potential of aerosolized *Salmonella* Enteritidis on colonization and persistence in broilers from d 3 to 21. Amrit Pal*^{GS 1}, Rachel Osborne¹, Andrea Urrutia¹, Alexandra Jackson¹, Matthew A. Bailey¹, Kenneth Macklin¹, Stuart Price³, R. J. Buhr², Dianna Bourassa¹; ¹Poultry Science, Auburn University, Auburn, Alabama, United States, ²USDA-ARS US National Poultry Research Center, Athens, Georgia, United States, ³College of Veterinary Medicine, Pathobiology, Auburn, Alabama, United States.

The presence of *Salmonella* in air of poultry houses has been confirmed through published research. Therefore, it is important to investigate the transmission of *Salmonella* to chickens via air. The present study aimed to evaluate the potential of different doses of aerosolized *Salmonella* Enteritidis inoculation, in day-old broilers, on colonization of ceca, liver/spleen, and trachea of broilers over time. For each of the three independent trials, a total of 112 1-d old birds were randomly divided into four groups (n=28/group). On d 1 of bird age, group 1 (control) was exposed to aerosolized sterilized saline and remaining groups were exposed to one of three doses, 10³ CFU/mL (group 2), 10⁶ CFU/mL (group 3), 10⁹ CFU/mL (group 4), of aerosolized *Salmonella* Enteritidis. Aerosolized exposure time was 30 min/group and was performed using a nebulizer. Nebulizer generated aerosol particles were <5 μm and the average nebulizing rate was 0.20 mL/min. Following nebulization, birds were reared in separate battery cages up to 21 d of age. On d 3, 7, 14, and 21 of age, ceca, liver/spleen, and trachea of 6 birds/group/trial were aseptically removed following euthanasia. Tissues were cultured for *Salmonella* prevalence and ceca for *Salmonella* counts (log₁₀ CFU/g). *Salmonella* counts were analyzed by two-way ANOVA with Tukey's HSD for means separation. Prevalence data were analyzed using Fisher's exact test. All sampled tissues from the control group were *Salmonella* negative. On sampling d 3 and 7, ceca *Salmonella* counts were highest from group 4 (5.14 and 5.11, P<0.0281). No differences for *Salmonella* counts were observed among groups on d 14 or 21 (P>0.5666). In group 2, ceca *Salmonella* counts increased (P=0.0188) from

d 3 (2.43) to d 7 (4.43) and then remained constant. Ceca *Salmonella* counts decreased over time for groups 3 and 4 (P ≤ 0.0005, 4.56 to 2.59 group 3, 5.14 to 2.81 group 4). For each tissue, *Salmonella* prevalence increased with increasing inoculum levels at all sampling timepoints (P<0.0213). *Salmonella* prevalence was low (0/18 to 4/18) and did not change over time in sampled tissues for group 2 (P>0.2394). For groups 3 and 4, *Salmonella* prevalence decreased over time in ceca (17/17 to 8/18 group 3, 18/18 to 12/18 group 4) and trachea (17/18 to 5/18 group 3, 18/18 to 14/18 group 4) (P<0.0483). For group 4, liver/spleen prevalence increased from d 3 (11/18) to d 14 (18/18) and then decreased at d 21 (10/18) (P=0.0015). Overall, this study demonstrated that *Salmonella* colonization and persistence in chickens following aerosol inoculation is dependent on *Salmonella* challenge levels and sample time post exposure.

Key Words: Salmonella, broiler, aerosol, tissues, poultry

254 Effect of inoculation dose on prevalence and concentration of *Salmonella* Reading in commercial turkey poults. Estefania Novoa Rama*^{GS 1}, Davis A. Fenster², Jasmine Kataria¹, Gaganpreet Sidhu¹, Sasikala Vaddu², Amanda Elisa Moller¹, Cortney Leone², Thiago Belem², Anju Singh², Rami A. Dalloul², Harshavardhan Thippareddi², Manpreet Singh^{1, 2}; ¹Department of Food Science and Technology, University of Georgia, Athens, Georgia, United States, ²Department of Poultry Science, University of Georgia, Athens, Georgia, United States.

Salmonella enterica serovar Reading (S. Reading) has been recently associated with multistate outbreaks in the United States and Canada through contaminated turkey products. This serovar exhibits a high potential for persistence and dissemination in turkey production facilities, yet more research is needed to characterize its behavior in live birds. The objective of this study was to determine the effect of S. Reading inoculation dose on cecal colonization and dissemination to internal organs following experimental challenge of young turkeys. Turkey poults (N=487) were assigned to four challenge groups corresponding to a S. Reading inoculation dose of 10², 10⁴, 10⁶, or 10⁸ CFU/mL (n=112/group), along with a non-inoculated control group (n=39). The birds were orally challenged on day 7 of age and euthanized on days 9 (n=50), 14 (n=314), and 21 (n=50). On sampling days, the ceca, liver, and spleen were aseptically removed for microbiological analysis. Ceca samples were collected from all turkey poults (n=414), liver and spleen samples were collected from 50 birds (n=150/organ). The prevalence and concentration of *Salmonella* were determined following the SalQuant procedure of the BAX® PCR System, along with conventional surface plating using XLD agar. One-way ANOVA and Chi-Square were used to evaluate statistical differences. Recovery of *Salmonella* was highest in the ceca (P ≤ 0.05). Turkey poults challenged with 10² CFU/mL had the lowest prevalence of *Salmonella* (P ≤ 0.05) and reached a maximum of 60% at 7 days post-challenge. In the

remaining groups, the prevalence ranged from 90 to 99%. Inoculation doses of 10^2 , 10^4 , 10^6 , and 10^8 CFU/mL resulted in 2.1, 3.3, 5.8, and 6.0 \log_{10} CFU/mL colonization of the ceca, respectively. Oral challenge with a dose of 10^8 CFU/mL resulted in the higher prevalence and persistence of colonization. The concentration of *Salmonella* in treatment groups 10^6 and 10^8 CFU/mL decreased to 3.3 and 4.6 \log_{10} CFU/mL after 28 days, while the prevalence remained similar. The opposite trend was observed in treatment groups 10^2 and 10^4 CFU/mL. Dissemination of *S. Reading* to internal organs was low, irrespective of inoculation dose, and the prevalence did not surpass 20% and 10% in the liver and spleen, respectively. Comparison of quantitative methods revealed that *Salmonella* detection rates and mean populations were higher when using SalQuant ($P \leq 0.05$). This study highlights the ability of *S. Reading* colonization of turkeys and identifies an optimum challenge dose to evaluate poultry production interventions in reducing its prevalence at pre-harvest.

Key Words: *Salmonella Reading*, turkey, colonization, ceca, challenge

255 Development of a protocol for early colonization of the chick intestinal tract. Laura Franco*^{GS 1}, Marcio Costa¹, Martine Boulianne², Eric Parent³; ¹*Biomedicine Veterinary, University of Montreal, St.-Hyacinthe, Quebec, Canada*, ²*Clinical Sciences, Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, Quebec, Canada*, ³*Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, Ontario, Canada*.

Introduction: Ideally, the chicken gastrointestinal tract should be colonized by bacteria that are important to ensure health and growth performances. First colonisers can influence immune system hence immunity. However, microbiota manipulation in chicks to obtain a stable bacterial population has had limited success. **Objective:** To develop a protocol of colonization of newly hatched chicks using different profiles of cecal microbiota. **Methods:** 240 Cobb eggs were divided into the following 4 treatment groups: a) Conventional: cecal content of commercial broiler chickens raised under conventional production system, b) Organic: cecal content of organically raised layer hens with open access to pasture, c) Autoclaved: autoclaved cecal content of organic layer hens and d) Control: no cecal content. The cecal content of 10 donor birds was diluted in 1L of saline, which was spread on eggs the day before hatching, given by gavage (1ml) after hatching and mixed with the drinking water. Ten birds per group were euthanized at day 2, 7, 14, 28 and 42. Next generation sequencing was used to characterize cecal bacterial communities. **Results:** Cecal microbiota composition at D7, D14, D28 and D42 was statistically different between treatments ($P < 0.001$) and more similar to the donor's bacteria. Time of sampling (age) also impacted the cecal microbiota ($P < 0.001$). **Conclusions:** This protocol was efficient to colonize chicks' ceca with the donors' cecal

microbiota. Age and source (donor) influenced the cecal microbiota of chickens during the entire grow out with initial colonization creating a stable and long lasting cecal microbiota. The protocol developed and the data generated in this study can both be used for the development of microbiome-based interventions to enhance performance and to prevent diseases in commercial flocks.

Key Words: microbiota, immunity, gavage, colonization

256 Effect of dietary resistant starch on cecal microbiota and metabolome in Pekin ducks. Simeng Qin*^{GS}, Keying Zhang, Xuemei Ding, Jianping Wang, Shiping Bai, Qiu Feng Zeng; *Animal Nutrition Institute, Sichuan Agricultural University, Chengdu, Sichuan, China*.

The microbial barrier within cecum plays an important role in protection against pathogens and maintain intestinal health in poultry. Alteration in gut microbial composition literally brings a wide variation of metabolites and metabolic pathways, which consequently exert a tremendous influence on host health. Currently, knowledge about the impact of dietary resistant starch on the link between cecal microbiota and metabolites is limited. In this study, Illumina HiSeq high-throughput sequencing and LC-MS/MS technology for untargeted metabolomics analysis were used to investigate the effect of raw potato starch (RPS, RS2) on microbial composition and metabolites in the cecum of Pekin duck. A total of 200 1-d-old male ducklings were fed (10 pens of 10 ducklings, on each diet) either basal diet without RPS (Control) or RS diet with 12% RPS for 21 days. Subsequently, samples of cecal contents were prepared for examination. All data analyses were performed using R and Python. The model construction was performed by using R package Vegan. The microbial results showed that RS group significantly elevated (1.2-fold) in Firmicutes abundance and Firmicutes/ Bacteroidetes ratio compared with the control group. Based on Lefse analysis, birds fed RS diet had significantly increased levels of Lactobacillales, Rothia, Erysipelotrichaceae, and Coprobacillus compared to basal diet-fed ducks. As for metabolomic analysis revealed that metabolic pathways, tryptophan metabolism, Steroid hormone biosynthesis, vitamin B6 metabolism, arginine and proline metabolism, tyrosine metabolism, mTOR signaling pathway, phenylalanine, tyrosine and tryptophan biosynthesis and FoxO signaling pathway were impacted by RS diet. And RS supplementation significantly upregulated the abundance of Kynurenic acid, Arachidonic acid, Equol, 5-Hydroxyindoleacetic Acid and 4-phenylbutyric acid, etc., while downregulated the Indolelactate, Indoleacetaldehyde, Creatine, Neu5Ac, Aflatoxin G1 and Glycerophospho-N-palmitoyl ethanolamine, etc. Furthermore, a Spearman's correlation analysis indicated that Lactobacillaceae was positively correlated with Ethylestrenol, Androstenedione, N-acetyl-L-cysteine, S-succinyl-dihydrolipoamide, Ethyl cinnamate, Cyanidanol, Homocarnosine, Imazaquin, Tryptophyl-4-hydroxyproline and kynurenic acid, etc., while negatively with Methional, Indole-3-lactic acid, Indole-3-

acetaldehyde, Malic acid, Glutaral, and 3-methylglutaric acid, etc. Accordingly, these results suggest that RS not only alters the composition of the gut microbial composition but also modulates the metabolomic pathway and metabolites, which may further affect the intestinal health of the host.

Key Words: resistant starch, cecal microbiota, metabolome, intestinal health, Pekin ducks

257 Role of microbiota on *Campylobacter jejuni* chicken colonization. Ayidh Almansour*^{GS 2, 1}, Ying Fu³, Tahrir Alenezi⁴, Mohit Bansal¹, Hong Wang¹, Xiaolun Sun^{1, 2}; ¹*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*, ²*Cell and Molecular Biology, University of Arkansas, Fayetteville, Arkansas, United States*, ³*Cell and Molecular Biology & Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*, ⁴*poultry science, University of Arkansas, Fayetteville, Arkansas, United States*.

Campylobacter jejuni is one of the worldwide prevalent foodborne bacterial pathogens mainly transmitted from poultry. However, few mechanisms are available on why *C. jejuni* colonizes chickens. In this study, we aimed to investigate the mechanism of transplanting microbiota on *C. jejuni* chicken colonization. Mouse specific pathogen free (SPF) microbiota was cultured on Brain Heart Infusion agar (BHI) and collected as SPF-Aerobe and SPF-Anaerobe. Birds raised on floor pens were colonized with 10⁸ CFU/bird SPF-Aerobe and SPF-Anaero at d 0 and infected with 10⁹ CFU/bird *C. jejuni* chicken isolate AR101 at d 12. Birds were sacrificed at day 28 to enumerate *C. jejuni* cecal colonization on selective *Campylobacter* plates. *C. jejuni* AR101 was co-cultured with SPF-Anaero and chicken anaerobic microbiota (Ch-Anaero) for examining the impact of microbiota on the pathogen *in vitro* growth at an inoculation ratio of 1:100, and 1:1000. As a result, we found that the SPF-Aerobe and SPF-Anaero microbiota reduced 91 % and 96 % of *C. jejuni* colonization in chicken ceca at day 28 (1.9x10⁵ and 8.5x10⁴ vs. 2.4x10⁶ CFU/bird, respectively) compared to infected alone birds. Notably, transplanted SPF-aerobe and SPF-Anaero increased Bacteroidetes (51.89 and 47.60% vs. 12.33%, respectively) compared to infected control birds. SPF-Anaero reduced *C. jejuni* growth by 99% (1.4 x10⁵, and 0 CFU/ml) for both 1:100, and 1:1000 inoculations, while Ch-Anaero reduced *C. jejuni* growth by 97% and 99% (5.6 x10⁵ and 0 CFU/ml) for the inoculations. Interestingly, SPF-Anaero and Ch-Anaero were comparable on inhibiting *C. jejuni* growth. In conclusion, SPF-aerobe and SPF-Anaero microbiota resisted against *C. jejuni* chicken colonization, while SPF-Anaero and Ch-Anaero reduced *C. jejuni in vitro* growth.

Key Words: Microbiota, c.jejuni, foodborne pathogen, bacterial colonization

258 Assessment of cecal cycling in broilers and turkeys. Olivia A. Wedegaertner*^{GS}, Chongxiao Chen, Frank Edens, Robert Beckstead; *Prestage Department of Poult. Sci. 100 (E-Suppl 1)*

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The ceca, in poultry, are two blind out-pockets of the GI tract located at the ileocecal junction. Small undigested feed particles, water, and urine are filtered into and fill the ceca through reverse peristalsis (watery stage), the diverse microbe population ferments the contents (frothy stage), the water is absorbed (pasty stage), and a cecal dropping is expelled about twice per day, emptying the ceca and restarting the cycle. The objective was to evaluate the cecal cycle stage of poultry at different ages and with different stressors (light and feed withdrawal (FW)) to understand how the poultry ceca cycles at various stages of production. A series of 5 studies were conducted. The first three studies [Study 1: 600 18-week-old turkeys; Study 2: 1,920 7-week-old broilers; and Study 3: 96 12-day-old broilers] involved observation of the cecal cycle stage of turkeys and broilers. Studies 4 and 5 were conducted to evaluate the effect of light and FW on broiler cecal evacuation. In study 4, 60 7-week-old broilers were evenly divided into two treatment groups (T1: light on (4-5am) and FW (7pm-7am); T2: light off and FW (7pm-7am)). In study 5, 75 7-week-old broilers were evenly divided into 3 treatment groups (T1: light on (3:30-6:30 am) and FW (6:30pm-6:30am); T2: light on (3:30-6:30 am) and no FW; T3: light off and FW (6:30pm-6:30am)). Birds in studies 4 and 5 were sampled at 7am and 6:30am, respectively, the cecal cycle stage was recorded for each bird. In study 1, 8 turkeys (1.33%) had empty ceca while 508 turkeys (84.67%) had ceca in the pasty stage, 31 of which (5.16%) were partially emptied. All broilers in study 2 had full pasty ceca. In study 3, 19 broilers (19.79%) had empty ceca while 72 broilers (75%) had ceca in the pasty stage, 11 of which (14.67%) were partially emptied. In study 4, all 60 broilers had full pasty ceca; 2 fresh cecal droppings were observed in the T2 pens. In study 5, there were 74 broilers (98.6%) with pasty ceca; 5 birds (20%) in T1 had pasty ceca that were partially emptied, no cecal droppings were observed; T2 had one bird (4%) with frothy ceca and 2 birds (8%) with a pasty, partially emptied ceca, no cecal droppings were observed. In conclusion, studies 1-3 suggest that broiler ceca stop completely emptying some time after 2 weeks of age and remain in the pasty stage. Turkey ceca continue cycling more than broiler ceca in the later stages of production. Studies 4 and 5 indicate that under these conditions light and FW play a minor role in the cycling of the ceca. Although more research needs to be done, our results indicate that cecal cycling changes throughout production which raises concerns of dysbiosis and contamination issues at the processing plant.

Key Words: ceca, microbial fermentation, broiler, turkey, cecal cycle

259 Reduction of cecal colonization and internal organ dissemination of emerging *Salmonella enterica* serovar Reading using a turkey-derived probiotic and a *Salmonella* Typhimurium vaccine in growing turkeys. Claire Peichel*^{GS 1}, Shijinraj

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Identification of uncommon *Salmonella* serovars in turkey production as etiological agents of foodborne outbreaks has alerted the industry to search for proactive measures to control these pathogens before outbreaks result. The 2017-2019 *S. Reading* outbreak through raw turkey products is an example of such emergence and warrants rapid control measures. This study investigated the antibacterial effects of a turkey-derived *Lactobacillus salivarius* probiotic and a live attenuated *S. Typhimurium* vaccine (AviPro Megan Egg) on cecal colonization and internal organ dissemination of *S. Reading* in growing turkeys. Thirty, day-old poult were allocated into one of 5 groups: negative control (NC), positive control (PC), vaccine (VAC), *L. salivarius* (LB), and the combination of the vaccine and *L. salivarius* (LB+VAC). Poults in VAC and LB+VAC groups were vaccinated upon arrival and boosters were given at 3 weeks of age and 2 days before *Salmonella* challenge at 6th week. The LB and LB+VAC groups were supplemented with 7 log₁₀ CFU/gal of *L. salivarius* through the drinking water every other day until the *Salmonella* challenge, and then every day until sample collection. All turkeys except those in the NC group were inoculated with approximately 5 log₁₀ CFU/mL of *S. Reading*. Seven days after inoculation, ceca, crop, liver, and spleen were collected from euthanized turkeys for *Salmonella* enumeration and enrichment. All cecal samples were homogenized and diluted and the appropriate dilutions were plated on Xylose Lysine Deoxycholate (XLD) agar and plates were incubated at 37°C for 24 hours before enumeration. Liver, spleen, and crop samples were enriched in selenite cysteine broth and streaked on XLD plates for *Salmonella* identification after enrichment. Each treatment group had 6 birds, and the study was repeated 3 times (N=90). Data were analyzed using the PROC-MIXED procedure of SAS and significance was detected at *P*<0.05. The results of the study indicate that all treatments were effective in reducing emerging *S. Reading* in growing turkeys. The PC group had ~3log₁₀ CFU/g *S. Reading* colonized in their ceca. Compared to PC, the VAC group resulted in the highest reduction across all studies (2.3 log reduction; *P*<0.05). LB resulted in 1.8 log₁₀ CFU/g reduction and the combination a log reduction compared to PC (*P*<0.05). All treatments significantly reduced dissemination of *S. Reading* to liver and spleen, and reduced recovery from crop. Results indicate that the turkey-derived *L. salivarius* and the *S. Typhimurium* vaccine are effective in reducing the cecal colonization and internal

organ dissemination of *S. Reading* in growing turkeys. (NIFA #2020-67017-30787; #2016-38420-25285)

Key Words: *Salmonella Reading*, *Lactobacillus*, Vaccination, Growing Turkeys, Alternatives to Antibiotics

260 In silico prediction of novel vaccine candidates to reduce *Campylobacter jejuni* in chickens. Sabin Poudel*^{GS 1}, Mark A. Arick II², Chuan-Yu Hsu², Wei Zhai¹, Anuraj Sukumaran¹, Aaron Kiess¹, Li Zhang¹; ¹Department of Poultry Science, Mississippi State University, Mississippi State, Mississippi, United States, ²Institute for Genomics, Biocomputing, and Biotechnology, Mississippi State University, Mississippi State, Mississippi, United States.

Campylobacter jejuni (*C. jejuni*) is a common food-borne pathogen that causes human gastroenteritis. Poultry products are considered as major reservoirs for *C. jejuni* and the consumption of contaminated poultry products is the main source of human infection. Controlling *C. jejuni* colonization in poultry is greatly needed to reduce the frequency of food-borne illness in humans. The current study aimed to identify the conserved surface-exposed antigens of *C. jejuni*, which could be used to develop subunit vaccines to reduce *C. jejuni* colonization in the poultry GI tract. The genome sequences of four *C. jejuni* isolates from retail broiler carcass and giblets were utilized to identify vaccine candidates by the reverse vaccinology strategy. Protein-coding sequences were predicted and annotated using Prokka v1.13. The predicted protein sequences were analyzed using Vaxign to determine the subcellular protein localization, transmembrane helix, and adhesion probability. VaxiJen predicted the protein antigenicity. Conservancy analysis of selected antigens was performed by local tblastn utilizing Geneious Prime v2020.2.2, which was analyzed against a database of 206 *C. jejuni* strains and 34 *C. coli*. Finally, blastp analysis was performed to confirm that the host *Gallus gallus* (taxid:9031) does not express the selected proteins. From the four *C. jejuni* strains evaluated (MS2005, MS2058, MS2074, and MS2169); 1,820, 1,933, 1,629, and 1,750 protein-coding sequences were predicted, respectively. Among these proteins, a total of 30 proteins were selected as they were predicted to be present in an outer membrane or extracellular matrix, with transmembrane helices ≤ 1 and an adhesion probability score ≥ 0.5. From four screened *C. jejuni* strains, VaxiJen prediction for antigenicity revealed, 27 out of these 30 proteins were antigenic (antigen score ≥ 0.5). Among the 27 antigenic proteins, 21 protein sequences were conserved (presence in > 80%) within the *C. jejuni* and *C. coli* strains. None of the proteins were expressed by the host *Gallus gallus*. Among the 21 conserved protein sequences obtained, five proteins were found in all four *C. jejuni* strains and one protein (FlgE) was found only in MS 2074 strain. Overall, six conserved proteins out of the initial 21 were identified as conserved protein antigens (Phospholipase A, MoMP or PorA, FlgE, FlhD, TonB, and Cj-cdtB). Out of the six conserved proteins, three novel antigens (Phospholipase A, TonB, and Cj-cdtB) were

identified, which have not been tested as vaccine candidates and have the potential for the development of efficient vaccines. In conclusion, this study identified novel and highly conserved vaccine candidates, which could be used to develop subunit vaccines.

Key Words: *Campylobacter jejuni*, reverse vaccinology, antigen selection, subunit vaccine, poultry

261 Prediction of fumonisins B₁ and B₂ through Near Infrared Reflectance Spectroscopy (NIRS) in distiller's dried grains with solubles (DDGS). Denize Tyska*^{GS 1, 2}, Daniel F. Soares¹, Giséle P. da Rosa¹, Magdiél A. Reghelin¹, Rodrigo d. Carvalho¹, Raul F. Marcon¹, Adriano O. Mallmann², Carlos A. Mallmann¹; ¹*Department of Preventive Veterinary Medicine, Federal University of Santa Maria, Santa Maria, Brazil*, ²*Pegasus Science, Santa Maria, Rio Grande do Sul, Brazil*.

Distiller's dried grains with solubles (DDGS) is the major by-product of biofuel plants that use corn as raw material. This cereal is commonly contaminated by toxic substances known as mycotoxins. *Fusarium* stands out among the various genera producing these toxins, as it includes the main producers of fumonisins (FBs). In poultry, ingestion of FBs triggers numerous negative effects upon animal performance. DDGS has been used as an alternative to protein sources in poultry diets due to its nutritional characteristics. As well as other mycotoxins, FBs present in corn are concentrated in DDGS during its processing, and mycotoxicological monitoring is essential before its use in feed formulations. Advanced methodological tools, such as the Near Infrared reflectance spectroscopy (NIRS), have been gaining ground in the Industry due to the speed in analysis of multiple parameters. Thus, this work aimed to develop a method for predicting FBs B₁ and B₂ in DDGS using NIRS in association with chemometric methods. One hundred and sixty-six DDGS samples were used to build the models. Two datasets were created: a calibration group, containing 132 samples, and an external validation group, including 34 samples. The material was received and promptly analyzed at the Laboratory of Mycotoxicological Analyses (LAMIC), Santa Maria (Brazil), by Liquid Chromatography Coupled to Tandem Mass Spectrometry (LC-MS/MS; reference method) throughout 2020; another fraction was used for optical data collection in order to build the spectra library. The spectra were obtained in a Foss XDS Rapid Content[®] Analyzer and the data were extracted and converted into a JCAMP file. The final spectral data were exported to conduct the chemometric analyses via the Unscrambler v.9.7 software (CAMO, Norway). FBs levels varied from 250 to 8,360 µg.kg⁻¹; mean value and standard deviation were 3,897 and 1,627 µg.kg⁻¹, respectively. The models were evaluated separately for FB₁ and FB₂. Partial least squares was the regression method applied in the models, using cross-validation. The calibration results for FB₁ and FB₂ were, respectively: correlation coefficient, 0.90 and 0.89; coefficient of determination, 0.80 and 0.79; root mean square error of prediction, 527 and 237; and

residual prediction deviation, 2.30 and 2.20. Values of the external validation dataset were compared with the levels obtained via LC-MS/MS and the Mann-Whitney test was applied. No statistical difference was found between the groups (p : 0.085), thus indicating a satisfactory predictive ability and confirming the potential of NIRS to predict FBs in DDGS.

Key Words: DDGS, by-products, fumonisins, NIRS, chemometric methods.

262 Modeling the thermal inactivation of *Salmonella* reduction in poultry feed in a lab-based water bath. Tim Boltz*^{GS 1}, Victoria Ayres², Cangliang Shen¹, Joe Moritz¹ ¹*West Virginia University, Morgantown, West Virginia, United States*, ²*Animal and Nutritional Sciences, West Virginia University, Morgantown, West Virginia, United States*.

Feed hygienics are of increasing concern for poultry producers in the effort to produce safe poultry products with little to no *Salmonella* contamination. This project included two studies to investigate kinetic parameters of *Salmonella* in poultry mash feed with various heating temperatures. In study one, two- and five-gram samples were inoculated with *Salmonella* Typhimurium and then submerged in a heated water bath set at 90C and internal temperature of feed was monitored with a thermocouple until the temperature reached 75, 80, and 85C. In study two, two-gram samples were inoculated with *Salmonella* Typhimurium and submerged into a water bath set at 75, 80, 85, 90, and 95C for 0 to 180 s. Counts of pathogens were analyzed on xylose-lysine-Tergitol-4 (XLT-4) with tryptic soy agar overlay. Thermocouple data demonstrated two-gram and five-gram samples achieved a 5-log reduction of *Salmonella* when internal temperature was heated to 80°C and 85°C, respectively. United States Department of Agriculture (USDA) IPMP-Global fit software was used to calculate D-values for Weibull and linear models for feed heated at five temperatures. D-value is defined as the time required at a given temperature to achieve a 1-log reduction of a pathogen of interest. Weibull model calculated D-values were 2.27, 3.67, 3.95, 4.68, and 7.63 s when heated at 95, 90, 85, 80, and 75C, respectively. Linear model calculated D-values were 6.70, 8.83, 12.05, 13.91, and 24.40 s when heated at 95, 90, 85, 80, and 75C, respectively. After analyzing the whole data using USDA-IPMP-Global fit software, each individual temperature dataset was further analyzed using GinaFit software. The "shoulder" impact was not observed in most of the thermal kinetics data except for feed heated at 90C. In the opposite, the "tail" effect was observed in all data except for the 90°C. As heating temperatures decreased from 95 to 75C, Double-Weibull and Biphasic models fit all the thermal data from 90 to 75°C well, indicating that the *Salmonella* generated two subpopulations with different thermo-resistance. Results from this study demonstrate that Weibull, Linear, Double-Weibull, and Biphasic models are appropriate for predicting thermal inactivation of *Salmonella* in poultry

feed. These data may support feed mill standards to reduce *Salmonella* contamination in feed.

Key Words: Salmonella, Feed, Thermal Inactivation, Feed Manufacture, Feed Microbiology

263 Identification and characterization of *Escherichia coli* isolates from poultry litter. Maryann Khong^{*1}, Nicolle Barbieri², Ashlyn Snyder¹, Anna Magnaterra¹, Shawna L. Weimer¹; ¹*Animal and Avian Science, University of Maryland, College Park, Maryland, United States*, ²*Population Health, University of Georgia, Athens, Georgia, United States*.

This study aimed to investigate the antibiotic resistance profile of *Escherichia coli* (*E. coli*) found in poultry litter to ampicillin, streptomycin, and erythromycin, as well as *E. coli* O serogroups and virulence genes. *E. coli* is a major cause of foodborne illnesses and a source for antibiotic resistance. *E. coli* can also negatively impact the welfare of broilers and cause significant economic losses for poultry farmers. Litter samples were collected from 16 pens housing broiler chickens (d53) and *E. coli* was isolated (n=48 isolates). Antibiotic resistance of *E. coli* isolates was determined by performing the Kirby Bauer Test. Digital images were taken of each plate. The diameter of each zone of inhibition (ZOI) was measured with a ruler (Live) and with images of the plates using ImageJ (Digital). O serogroup and genotyping confirmation of *E. coli* with 16S RNA was performed by PCR. Also, a multiplex PCR was performed for the presence of 9 virulence genes indicative of avian pathogenic *E. coli* (APEC). Student's t-tests were used to determine the effects of measurement method on the cumulative ZOI for all plates and for each antibiotic as well as the effect of antibiotics on the ZOI within each method. Each ZOI was categorized into 1 of 3 antibiotic susceptibility categories (susceptible, intermediate, resistant). There was no difference in the ZOI of the Live and Digital measures across all plates (P = 0.23). Within each antibiotic, there was no difference in the ZOI of ampicillin and streptomycin, but the Digital ZOI of erythromycin was significantly greater (P=0.003) compared to Live (12.8 vs. 10.2 mm, respectively). Independent of the measurement method, the ZOI for ampicillin was greater (P0.002) than streptomycin and erythromycin. The identified O serogroups of the *E. coli* isolates were O15, O75, O78, and O91. Among the *E. coli* isolates, the highest resistance was to erythromycin (50%), followed by streptomycin (34.1%) and ampicillin (18.2%). There were five instances of multidrug resistance to all three antibiotics seen in some isolates with O serogroups O78 and O15. Of the genes indicative of APEC-like *E. coli*, there were five unique combinations. Within the APEC-like *E. coli* isolates, 12 exhibited resistance to one or more of the antibiotics. These isolates all contained plasmid genes *ironN*, *ompTp*, and *hlyF*. None of the APEC-like *E. coli* isolates exhibiting antibiotic resistance contained chromosomal genes. Consistent monitoring for *E. coli* antibiotic resistance would provide the necessary depth and breadth of information to

develop effective solutions to this global public health problem.

Key Words: Virulence genes, O serogroup, Kirby Bauer, Avian pathogenic *E. coli*, Broiler

264 Efficacy of Trans-cinnamaldehyde nanoemulsions in inactivating *Salmonella* Enteritidis on shelled eggs and chicken skin. Jodie T. Allen^{* GS 1}, Brindhalakshmi Balasubramanian¹, Kimberly Rankin¹, Trushenkumar Shah Sr.¹, Annie M. Donoghue², Indu Upadhyaya^{4 1}, Yangchao Luo³, Abhinav Upadhyay¹; ¹*Animal Science, University of Connecticut, Storrs, Connecticut, United States*, ²*Poultry Production and Product Safety Research, University of Arkansas, Fayetteville, Arkansas, United States*, ³*Nutritional Sciences, University of Connecticut, Storrs, Connecticut, United States*, ⁴*Department of Extension, University 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 or carcass wash treatment. This study investigated the efficacy of TC nanoemulsions (TCNE) as dip treatments for reducing SE on shelled eggs and chicken skin. 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 µL; attachment time 60 min) with a 4-strain mixture of SE (10⁷ CFU/mL) followed by dipping in sterile deionized water (control) or water containing TC, or TCNE at 0.01, 0.02, 0.03, 0.06, 0.12, 0.24, 0.48% for 1, 3, or 5 min at 34°C. Similarly, chicken skin (4 x 4 cm) was spot inoculated with SE (200 µL; attachment time 120 min), followed by dipping in water (control), or water containing TC or TCNE-Tw.80 (0.5, 1, 2, 5%) for 15, 30, or 60 min at 4°C. Post washing, the population of surviving SE on eggshell or chicken skin was enumerated on XLD agar. 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 98-140 nm and zeta potential range of -8 to -40 mV. In baseline eggs (SE inoculated, not washed), ~ 6.3 log CFU SE/egg were recovered. After washing with water for 5 min (control), ~ 4.3 log CFU SE/egg were recovered. When washed with TC, only the highest concentrations (0.12, 0.24, 0.48%) were effective in reducing SE by ~ 1.7, 2.7, 2.3 log CFU/egg, respectively, as compared to control by 1 min (P<0.05). In contrast, TCNE-Tw.80 treatments (0.06, 0.12, 0.24, 0.48%) were effective in killing SE by at least 2 to 2.5 log CFU/egg as early as 1 min (P<0.05). TCNE-Tw.80 and TCNE-GAL at 0.24, and 0.48% were the most effective treatments and reduced SE to below detection limit (3 log CFU/egg) by 1 min of treatment (P<0.05). In baseline

chicken skin, ~ 7.6 log CFU SE/sample were recovered. After washing skin with water for 60 min, ~ 7.4 log CFU SE/sample were recovered. TC was not effective in reducing SE on skin when compared to controls. TCNE-Tw.80 treatments (1, 2, 5%) were effective in killing SE by ~ 1.2, 1.4, 2 log CFU/sample, respectively, as compared to control, by 15 min (P<0.05). Results demonstrate the efficacy of TCNE against SE as an antimicrobial wash on shelled eggs and chicken skin. (Funded by USDA-ARS, ATA research #58-60022-8-006).

Key Words: Trans-cinnamaldehyde, Salmonella, nanoemulsions, food-grade emulsifiers, poultry products

265 Effect of Trans-cinnamaldehyde, Eugenol and Carvacrol on *Salmonella* Enteritidis proteome critical for colonization in chickens. Trushenkumar M. Shah*^{GS1}, Brindhalakshmi Balasubramanian¹, Indu Upadhyaya², Kumar Venkitanarayanan¹, Abhinav Upadhyay¹; ¹Department of Animal Science, University of Connecticut, Willimantic, Connecticut, United States, ²Department of Extension, University of Connecticut, Willimantic, Connecticut, United States.

Salmonella Enteritidis (SE) is a foodborne pathogen of global concern that causes severe diarrhea in humans. Chickens act as a reservoir host for SE, wherein the pathogen colonizes the ceca leading to carcass contamination during slaughter and subsequent human infections. Several multi-drug resistant SE strains have been isolated from poultry and poultry products fuelling the research for developing antibiotic alternatives for controlling SE in chickens. Trans-cinnamaldehyde (TC), Eugenol (EG) and carvacrol (CAR) are Generally Recognized as Safe status plant-derived compounds that have been reported to exert significant anti-*Salmonella* efficacy in chickens. However, the underlying molecular mechanisms of their action are still unclear. This study investigated the effect of sub-inhibitory concentrations of TC, EG and CAR on the proteome profile of SE, especially the expression of proteins critical for colonization in chickens. Sub-inhibitory concentrations (SICs; compound concentrations below the minimum inhibitory concentrations that do not affect bacterial growth) of TC, EG and CAR against SE-31 strain (isolated from chickens) were determined by standard growth curve assay. For proteome profiling, SE-31 was cultured either in the presence or absence (control) of SICs of phytochemicals for 24 h at 37°C followed by SDS-PAGE based protein extraction. Extracted proteins were quantified and subjected to LC-MS/MS analysis followed by matching MS/MS spectra to *Salmonella* Enteritidis PT4 (Uniprot reference proteome ID UP00000613) and analysis using Scaffold software version 5. Differentially expressed proteins between samples were analyzed using Student's t-test (P<0.05). The sub-inhibitory concentration of TC, EG and CAR that did not inhibit SE growth as compared to control was 0.01% (~0.75 mM), 0.01% (~0.6 mM) and 0.008% (~0.5 mM), respectively. Approximately 1440 proteins

were identified by LC-MS/MS analysis of which ~ 170 proteins were down-regulated and ~100 proteins were upregulated by the phytochemical treatments. All phytochemical treatments down-regulated critical virulence proteins contributing to PSI-1 type III secretion system (SipA, SipB, SipC), motility (SefB), cellular metabolism (GlpB, MetK, SpeF), and polymyxin resistance (ArnA) (P<0.05). The expression of proteins involved in stress response or cellular transport (GroL, TolC, AcrA) was upregulated by phytochemical treatments (P<0.05). Phytochemical specific change in protein expression was also observed for several SE proteins. Overall, these results delineate the prospective mechanism of action of TC, EG and CAR against SE and provides a basis for their combinatorial application.

Key Words: Salmonella Enteritidis, Colonization, Chickens, Phytochemicals, Proteome

266 Establishing on-site diagnostic procedures for the detection of *Campylobacter jejuni* in poultry. Mackenzie A. Ripper*^{UG}, Sabin Poudel, Aaron Kiess, Li Zhang; Department of Poultry Science, Mississippi State University, Mississippi State, Mississippi, United States.

Campylobacter jejuni (*C. jejuni*) is the leading cause of foodborne illness in the United States. Poultry is responsible for up to 80% of the human *C. jejuni* infections, therefore controlling *C. jejuni* in poultry is greatly needed to reduce the burden of foodborne illness in humans. Rapid and accurate detection of *C. jejuni* on-site is one of the first steps to minimizing the spread of this pathogen. Loop-mediated isothermal amplification (LAMP) is a nucleic acid-based amplification technique that uses a constant temperature to detect this pathogen. Compared to the polymerase chain reaction (PCR), which is costly and requires specialized equipment, LAMP offers a simple, sensitive, specific, and low-cost assay, which does not require specialized equipment and can be performed on-site. The objective of this study was to develop an on-site rapid detection assay for the determination of *C. jejuni* utilizing the LAMP method. In this experiment, pooled fecal samples were randomly collected from a commercial broiler farm, and 20 g fecal samples were homogenized with 35ml of Phosphate-buffered saline (PBS) in sterile Whirl-Pak filter bags. The filtered samples were centrifuged to collect fecal pellets for DNA extraction. A *C. jejuni* ATCC 33560 cell pellet was homogenized with PBS and adjusted to make 1.44×10^7 CFU/ml and was then serially diluted from 1.44×10^7 to 1.44×10^3 CFU/ml. One ml fecal samples were spiked with 100 µl of the *C. jejuni* ATCC 33560 serial dilution. Spiked fecal samples were used to extract DNA using a Thermo Scientific GeneJET Genomic DNA Purification Kit following the manufactures' protocol. Obtained DNA samples were filtered using Zymo-Spin III-HRC Filter columns to enhance the purity. All LAMP assays were performed in a 25 µl reaction mixture containing WarmStart Colorimetric LAMP 2X Master Mix, primers, and template DNA at an isothermal temperature of 65 C. DNA samples

spiked with the 1.44×10^7 CFU/ml of *C. jejuni* showed color change (pink to orange) within 20 min, indicating positive results. However, samples spiked with 1.44×10^3 CFU/ml of *C. jejuni* did not change color until 50 min, indicating that the LAMP assay cannot determine the low concentration of *C. jejuni* presence in fecal samples. In conclusion, the LAMP assay was rapid, simple, and easy to handle, but it requires pure DNA to generate a positive result, which makes it challenging to perform on-site detection. In the future, a simple sample processing protocol is required for the on-site application of this LAMP assay, as well as quantitative analysis needs to be achieved to verify the sensitivity and specificity of the LAMP assay to the standard PCR assay.

Key Words: Loop-mediated isothermal amplification, Poultry, Nucleic acid-based amplification, Campylobacter, jejuni

267 Withdrawn.

268 Effect of Cetylpyridinium Chloride (CPC) on the microbiome of *Salmonella* Typhimurium and *S. Infantis* inoculated chicken skin. Elena G. Olson*^{GS 1}, Dana K. Dittoe¹, Lindsey A. Wythe¹, Zachary G. Lawless³, Lindsey Perry², Steven C. Ricke¹; ¹*Meat Science and Animal Biologics Discovery, Animal and Dairy Sciences, University of Wisconsin-Madison, Verona, Wisconsin, United States*, ²*Safe Foods, North Little Rock, Arkansas, United States*, ³*Computer Science and Computer Engineering, University of Arkansas, Fayetteville, Arkansas, United States*.

As poultry continues to be a significant contributor of salmonellosis in the US, especially with the emergence of pESI-like megaplasmid among *Salmonella* Infantis strains isolated from poultry meat, the poultry industry is tasked with determining effective mitigation strategies. Therefore, the objective was to evaluate the effects of peroxyacetic acid (PAA) and cetylpyridinium chloride (CPC) on the microbiota of chicken skin inoculated with either *Salmonella* Typhimurium or *S. Infantis*. Chicken breast skins (4 x 4 cm; N = 100, n = 10, k = 5) were inoculated with either *Salmonella* Typhimurium or *S. Infantis* and allowed to adhere 4 °C for 30 min for a final attachment of 10^8 CFU/gram. Skins were shaken for 30 s to remove the excess fluid, with remaining bacteria being considered firmly attached. The following treatments were applied as 30 s dips in 50 mL: no inocula-no treatment control (NINTC), no treatment control (NTC), tap water (TW), TW+500 ppm PAA (PAA), or TW+0.5% CPC (CPC). Excess fluid was shaken off (30 s) and skins were rinsed in neutralizing buffered peptone water and stomached for 1 min. Genomic DNA of rinsates was extracted, and the 16S rDNA was sequenced (Illumina MiSeq platform). Microbiota data were filtered and aligned using the QIIME 2-2020.2 pipeline, with data considered significant at $P \leq 0.05$ for main effects and $Q \leq 0.05$ for pairwise differences. Results demonstrated that there was

significant interaction of treatment and inocula ($P < 0.05$) for Alpha Diversity metrics, but not for any Beta Diversity ($P > 0.05$). Skin inoculated with *S. Typhimurium* had a more even and rich microbiota than those inoculated with *S. Infantis* (Pielou's and Shannon's; $P < 0.05$). Among Bray Curtis and Weighted Unifrac, there were distinct differences in abundances and phylogenies between the inoculum administered and differences between PAA treated skins and of the controls ($P < 0.05$). Using ANCOM, the analysis of microbiomes, the inoculation of skins with *S. Typhimurium* resulted in an increased relative abundance of *Campilobacterota* and *Cyanobacteria* compared to those inoculated with *S. Infantis*. In addition, using ANCOM, the treatment of skins with CPC and PAA had the lowest relative abundance of *Proteobacteria* compared to all other treatments with a composition resembling NINTC treated skins. In conclusion, both PAA and CPC are capable of altering the microbiota of chicken skin when inoculated *Salmonella* (Typhimurium vs Infantis), with serovar differences being observed.

Key Words: poultry, 16S rDNA, Salmonella Infantis, peroxyacetic acid, cetylpyridinium chloride

269 Reduction of *Salmonella* Infantis on skin-on, bone-in chicken thighs by Cetylpyridinium Chloride application and the impact on the skin microbiota. Lindsey A. Wythe*^{GS 1}, Dana K. Dittoe¹, Kristina M. Feye², Elena G. Olson¹, Lindsey Perry³, Steven C. Ricke¹; ¹*Meat Science and Animal Biologics Discovery, Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, Wisconsin, United States*, ²*Food Science, University of Arkansas, Fayetteville, Arkansas, United States*, ³*Safe Foods, North Little Rock, Arkansas, United States*.

Salmonella Infantis has been the etiological agent of numerous foodborne outbreaks of non-typhoidal *Salmonella*. As such, there is an emergent need to mitigate *Salmonella* Infantis among poultry and poultry products. Thus, this study evaluated the efficacy of cetylpyridinium chloride (CPC) versus peroxyacetic acid (PAA), the standard poultry processing antimicrobial, on bone-in, skin-on chicken thighs for the reduction of *Salmonella*, as well as the changes in the microbiota. A total of 100 skin-on, bone-in chicken thighs (2 trials, 0 and 24 h, k = 5, n = 5, N = 50) were inoculated with 10^8 CFU/mL of a nalidixic acid (NA) resistant strain of *S. Infantis* for an attachment of 10^6 CFU/g. Inoculated thighs treated with 400 mL of the following 20 s part dips: a no inoculum, no treatment control (NINTC); inoculated, no treatment control (NTC); tap water (TW); TW+CPC; TW+PAA. Following, thighs were rinsed in 150 mL of nBPW, and the rinsates were collected downstream analyses. Rinsates spot plated for *Salmonella* and aerobic bacteria (APC). Corresponding plate counts were \log_{10} transformed and analyzed using a mixed-effects model (mixed effect = trial) with means separated using Tukey's HSD ($P < 0.05$). The genomic DNA of the rinsates was extracted, and the V4 region of the 16S rDNA was sequenced on an Illumina MiSeq.

Microbiota data were filtered and aligned using QIIME 2, with data considered significant at $P \leq 0.05$ for main effects and $Q \leq 0.05$ for pairwise differences. Treatment x time interactions were observed for both *Salmonella* and APC ($P < 0.05$). The treatment of thighs with PAA and CPC was capable of reducing *Salmonella* (5.19 and 5.38 Log_{10} CFU/g at 0 h and 4.96 and 4.29 Log_{10} CFU/g at 24 h) and APC (5.20 and 5.77 Log_{10} CFU/g at 0 h and 5.59 and 4.56 Log_{10} CFU/g at 24 h) in respect to the controls. Numerically, thighs treated with CPC had less *Salmonella* (4.29 Log_{10} CFU/g) and APC (4.56 Log_{10} CFU/g) at 24 h than all other treatments ($P > 0.05$). Differences in α or β diversity were not consistently observed between treatments; however, in trial 2, the NTC treated thighs were different than those treated with CPC ($P < 0.05$; $Q < 0.05$). In both studies, using ANCOM, the analysis of microbiome compositions, thighs were different in the relative abundances of *Proteobacteria* ($W > 2$; $P < 0.05$), with NINTC having less abundance of *Proteobacteria*. As well, ANCOM revealed shifts due to treatment or treatment x time at both the phylum and order levels ($P < 0.05$). In conclusion, the treatment of skin-on poultry parts with CPC may reduce the risk of foodborne outbreaks caused by *Salmonella* Infantis.

Key Words: poultry, microbiota composition, *Salmonella* Infantis, peroxyacetic acid, cetylpyridinium chloride

270 *Salmonella* Enteritidis decontamination on eggshell surface by High Voltage Atmospheric Cold Plasma. Aparajitha Sudarsan, Alba E. Illera*, Vanessa R. Souza, Kevin Keener; *School of Engineering and Physical Sciences, University of Guelph, Guelph, Ontario, Canada.*

The primary objective of this study is to investigate the effect of a Dielectric Barrier Discharge High Voltage Cold Plasma (HVACP) parameters on *Salmonella* Enteritidis reduction. Poultry eggs and their products are highly susceptible to pathogenic microorganisms, particularly, *Salmonella* spp., causing 1.2 million cases of serious illness in the United States alone and costing \$365 million for medical intervention. Their ability to persist through challenging growth conditions makes them particularly hard to eliminate from food surfaces such as eggs. Cold plasma, commonly called the ‘fourth state of matter’, is an ionized cocktail of reactive molecules and free radicals produced when a gas is passed through two electrodes with a sufficient potential difference to cause its breakdown and ionization. This reactive gas chemistry is the driving force behind microbial decontamination observed on various matrices, including and not limited to foods. In the current study, eggshell surfaces were inoculated with an initial load of 9 log CFU/mL of *Salmonella* Enteritidis and were subjected to HVACP under different treatment conditions. A number of variables such as treatment time (2-15 minutes), post-treatment hold time (0-24 hours), mode of exposure (direct/indirect), applied voltage (80-100 kV), gas used (Air/MA65) and relative humidity (0-80%) were tested for in triplicates and screened for their effectiveness.

Upon conducting Plackett-Burman screening analysis with a 95% confidence, it was observed that all variables tested had a significant ($p < 0.05$) and positive effect on *Salmonella* reduction while some of them such as the post-treatment time and voltage had a much more pronounced effect on the decontamination. Briefly, higher voltages, longer treatment, and post-treatment times under indirect plasma exposure resulted in >5 log CFU reductions, especially at higher humidity. For example, a 100 kV, 2 minutes treatment, and 24 hours post-treatment hold at 80% RH was a highly effective combination for achieving >5 log CFU reduction. Air was found to be the most effective working gas than MA65 (65% Oxygen, 30% Nitrogen, 5% CO_2) indicating a combination of reactive oxygen species (ROS) like ozone, OH^\cdot , H_2O_2 along with reactive nitrogen species (RNS) such as oxides of nitrogen, nitrates, nitrites and nitric acid that contribute towards effectively eliminating these pathogenic microorganisms from egg surfaces. Additional tests will be conducted to study the effects of plasma treatment on egg quality. In conclusion, HVACP is a safe and promising technology for achieving high standards of food safety sustainably with low energy inputs.

Key Words: cold plasma; salmonella; eggshells; decontamination; antimicrobial

271 Supply-chain temperature abuse of tray-packed raw chicken on pallets can influence spoilage at retail level. Charles B. Herron*^{GS 1}, Amit Morey², Aftab Siddique³, Laura J. Garner¹, Indira Medina¹, Peyton Williams¹; ¹*Poultry Science, Auburn University, Auburn, Alabama, United States*, ²*Poultry Science, Auburn University, Auburn, Alabama, United States*, ³*Poultry Sciences, Auburn University, Auburn, Alabama, United States.*

Objective: To demonstrate the effects of temperature abuse on spoilage of raw chicken at retail level. **Experimental Design:** An experiment was designed to simulate the repeated temperature abuse of raw chicken during supply-chain and study its effects on spoilage of chicken. **Materials and Methods:** A commercially produced pallet of tray-packed boneless skinless chicken breast (4 layers x 5 boxes per layer x 24 tray packs per box) was subjected to a cyclic temperature abuse pattern (2-hours 4 C and 2-hours at 22 ± 2 C) for 24-h. Temperature profiles of a representative fillet and air in each box was recorded using thermocouples and thermal images of the pallet were taken. Microbial sampling ($n = 1$ fillet/tray x 2 random trays/box x 2 boxes/layer x 4 layers) was performed by rinsing individual fillets with buffered peptone water (50 mL), serially diluting, and spread plating on duplicate plates on two sets of standard methods agar plates. Each set of plates was incubated at 37 C for 24-48-h and 4 C for 10 days to estimate aerobic plate counts (APC) and psychrotrophic plate count (PSY) (log CFU/mL of rinsate). After 24-h temperature abuse, boxes ($n=2$) with highest temperature abuse were pooled for shelf-life estimation and compared to an unabused box at 4 C for

8 days. Microbiological sampling (n=1 fillets/random tray x 3 trays x 2 treatments) was conducted every 2 days as above to determine the APC and PSY plate counts and was reported as log CFU/mL of rinsate. Fillets crossing the APC level of 7 log CFU were considered spoiled. *Statistical Analysis:* One-way ANOVA with Tukey's HSD ($p < 0.05$) was used to determine differences in the microbiological counts. APC and PSY growth trends were analyzed using regression models. *Results:* Layers 1 and 4 had a higher temperature increase than layers 2 and 3 on the pallet. The APC and PSY concentrations stayed consistent over the 24-h and ranged from 1-2 log CFU/mL of rinsate. During the shelf-life study, the APC and PSY of temperature abused

fillets increased to 7 logs (spoilage level) at 4.8 days and 3.8 days, respectively, compared to 7 days and 5.2 days, respectively for the control samples. Linear regression models for the APC growth trends ($r^2 = 0.87$ and 0.96 for temperature abuse and control, respectively) can predict spoilage. No significant difference was observed in APC ($p = 0.0513$) but was observed in PSY ($p = 0.0388$) when comparing 0-h and 24-h. Tukey's HSD showed no significant differences. *Conclusions:* Shelf-life of chicken breast reduced 2-days after simulated supply-chain cyclic temperature abuse indicating the significance of collecting time/temperature data during supply-chain.

Key Words: Temperature Abuse, Spoilage, Shelf-life, Cold Chain, Psychrotroph

Physiology and Reproduction

272 No presentation materials submitted.

273 Delayed feeding of newly hatched broiler chicks alters hepatic and adipocytic exosomal miRNA secretion and cross-tissue regulation. Julie Hicks*, Hsiao-Ching Liu; NC State University, Raleigh, North Carolina, United States.

Hepatic fatty acid oxidation of yolk lipoproteins provides the main energy source for chick embryos. Post-hatching these yolk lipids are rapidly exhausted and metabolism switches to a carbohydrate-based energy source. We recently demonstrated that a large number of microRNAs (miRNAs) are key regulators of hepatic and adipocytic metabolic pathways during this metabolic switching. MiRNAs are small non-coding RNAs which post-transcriptionally regulate gene expression in most eukaryotes. To further elucidate the roles of miRNAs in the metabolic switch we used delayed feeding for 48 hours post-hatching to impede the hepatic metabolic switch in Ross 708 broilers. We then used RNA-seq (Illumina) to identify transcription differences between embryos at 18 days of incubation (E18) and two-day-old chicks (D2) which were given access to feed (FED) or not provided feed (DLY). We then combined this with our miRNA expression data to further define the roles of miRNAs in regulating the metabolic switch. Finally, we determined if, broiler hepatocytes and adipocytes have the potential to release miRNAs via small lipid vesicle, termed exosomes. For RNA-seq data, a gene was defined as differentially expressed if it had a ≥ 2 -fold and $FDR \leq 0.05$; CLC-exact test (based on Robinson and Smith method). A total of 880 genes ($n=6$; fold-change ≥ 2 ; $FDR \leq 0.05$) were found to be differentially expressed in the liver between D2 broilers that were delayed access to feed for 48 hrs after hatch (DLY) and broilers fed-from-hatch (FED). Heat-map analysis revealed very distinct expression patterns between the two groups. Between FED birds and E18 embryos 2,430 genes were differentially expressed ($n=6$ fold-change ≥ 2 ; $FDR \leq 0.05$), and between DLY birds and E18 embryos 1,979 genes were differentially expressed ($n=6$ fold-change ≥ 2 ; $FDR \leq 0.05$). The top canonical pathways for differentially expressed genes unique to the DLY:E18 comparison are Aldosterone Signaling, Pregnenolone Biosynthesis, and the Unfolded Protein Response. The top canonical pathways for differentially expressed genes unique to the DLY:FED comparison include Cell Cycle Control of Chromosomal Replication and Role of BRCA2 in DNA Damage Response. These results indicate that even a short period of starvation after hatching induces metabolic disturbances and organ damage. Using RT-qPCR ($n=6$, ANOVA), we found that both hepatocytes (e.g. miR-30a) and adipocytes (e.g. miR-449c) can secrete miRNAs via exosomes. We further found that cross-treatment with exosomes significantly (RT-qPCR; $n=6$, $p \leq 0.05$; ANOVA) altered the expression of miRNA-targeted metabolic genes associated with lipid

metabolism and oxidative stress.

Key Words: broiler, metabolism, RNA-seq, microRNA, gene regulation

274 Sex differences in dose dependent release of both corticosterone and cortisol, and in heterophil to lymphocyte ratios in response to ACTH in the adult Pekin duck. Victoria K. Tetel^{*UG¹}, Brooke E. Van Wyk², Gregory S. Fraley¹; ¹Animal Sciences, Purdue University, West Lafayette, Indiana, United States, ²Biology, Hope College, Holland, Indiana, United States.

More often, poultry scientists are utilizing direct and indirect measures of stress hormones to monitor bird welfare. However, it has been clear that our understanding of the avian hypothalamic-pituitary-adrenal axis (HPA) is insufficient as evidenced by the many conflicting reports regarding stress responses, such as transportation stress, in poultry. It has long been assumed that the poultry HPA functions similarly to that of mammals. However, we now know that there are considerable differences in the avian HPA. In addition to corticotropin releasing hormone, the avian brain utilizes a second neurohormone, arginine vasotocin, and both are released from the brain to stimulate pituitary corticotropes (ACTH). Both glucocorticoids (GC) are synthesized from a common pathway that begins with cholesterol and pregnenolone. The synthesis of one of the glucocorticoids does not depend upon the synthesis of the other. The purpose of our study was to test the hypothesis that ACTH will stimulate both corticosterone and cortisol. To test this hypothesis, we injected intramuscularly artificial ACTH (cosyntropin; 0.0625, 0.031, 0.016 mg/kg or saline as control) into adult drakes and hens ($N = 10$ /sex/dose). Both glucocorticoids (GC) were assayed in serum using previously verified ELISAs. Blood smears were also assessed for heterophil to lymphocyte ratios (HLR). Data were analyzed by repeated measures 3-way ANOVA with Fishers PLSD as an ad hoc test. We observed that both GC were secreted in significantly ($p = 0.0002$) different patterns in a dose-dependent manner compared to controls, and that there was a significant ($p = 0.0001$) sex difference in both GC compared to saline controls. Further, we observed that the high dose ACTH elicited a significant ($p = 0.004$) sex difference in the HLR response compared to controls. Our data suggest that ducks, at least, may utilize more than just corticosterone for physiological homeostasis. Further, the time course of stressor to release of GC and subsequent HLR response may be dependent upon sex. More detailed analyses of the HPA is necessary in all poultry species to better understand stress responses as we utilize biological bases for welfare assessments.

Key Words: corticosterone, cortisol, ACTH

275 Acute injections of either corticosterone or cortisol elicit differential effects on heterophil to

lymphocyte ratios in a sex-dependent manner in Pekin ducks. Victoria K. Tetel*^{UG}, Sara Tonissen, Mallory Swanson, Gregory S. Fraley; Animal Sciences, Purdue University, West Lafayette, Indiana, United States.

Poultry scientists have utilized both direct and indirect measures of stress hormones for monitoring the state of avian welfare. However, it is now known that our current understanding of the avian hypothalamic-pituitary-adrenal axis (HPA) is insufficient following studies that have shown conflicting reports of avian stress responses, such as transportation stress. For decades, it has been assumed that the mammalian and avian HPA function similarly to one another. Now we know that there are considerable differences between the two, with one being the neurohormones involved. Originally, it has been assumed that the predominate glucocorticoid (GC) in birds was corticosterone, but recent studies have suggested that both corticosterone and cortisol are secreted in response to ACTH. GC release is associated with an increase in blood heterophils due to increased migration from the lymph nodes and a decrease in lymphocytes due to marginalization. Both of these factors account for an increase in heterophil to lymphocyte ratios (HLR). The goal of this project was to determine the effect of each GC on HLR over time. To achieve this, we intramuscularly injected 2.0 mg/kg of each GC, 0.5 mg/kg for the low-dose cortisol treatment, or safflower oil as vehicle control. Blood was collected at the prior to IM injections and blood collected three more times at every hour. Blood smears were also collected to assess HLR at the same four time points. HLR assays were completed by avian pathologists from an independent lab who were unaware of the treatments. Data were analyzed by 3-way repeated measures ANOVA with a $p < 0.05$ considered significant. We found significant sex ($p < 0.001$) x treatment ($p < 0.001$) x time ($p < 0.001$) effects with significant interactions ($p = 0.0055$). In hens, both GC resulted in significant increase in HLR at 1 hour after injection compared to controls. In drakes, however, both GC showed a significant increase in HLR but not until 2 hours after injection. The low dose cortisol had no significant effect on HLR in either sex. Combined with the ACTH dose response effects of each GC, these data suggest that sex differences need to be considered when assessing duck welfare, and that cortisol may play a role in the HPA axis in ducks in a sex-dependent manner.

Key Words: corticosterone, cortisol, sex differences

276 Relationship between broiler breeder eggshell cuticle deposition and incubation parameters. Luis P. Avila*^{GS}, Kelly M. Sweeney, Jeanna Wilson; Department of Poultry Science, University of Georgia, Athens, Georgia, United States.

Eggshell cuticle, predominantly composed of glycoproteins, provides antimicrobial and protective characteristics. It is unclear what factors contribute to cuticle deposition and if this proteinic layer could potentially improve incubation parameters. The objectives

of this study were to determine if broiler breeder hen age and performance parameters have an impact on eggshell cuticle, and if cuticle deposition contributes to an improved hatch of fertile eggs. Cobb 700 broiler breeder pullets ($n = 430$) were reared in floor pens up to 21 wk of age and fed a common starter diet through 3 wk of age. A common grower diet was fed to birds from 4 to 21 wk using a restricted skip-a-day feeding program. Pullets were then moved to laying pens ($n = 43$ per pen; $n = 10$ pens) with 4 Cobb Vantage males in each pen. A common laying diet was fed when hens reached 5 % egg production after 25 wk of age. Hens were feed-restricted and fed a common daily feed allowance depending on body weight and egg production goals. Broiler breeder hen egg production, egg weight, egg mass, and shell quality were determined through wk 50 of age. Eggs were incubated 6 times (29, 33, 37, 41, 45, and 49 wk of age) to determine hatch of fertile percentage and embryonic mortality diagnosis. In addition, pre and post-stain eggshell reflectance ($n = 100$ eggs per wk) was determined using a portable spectrophotometer to calculate eggshell cuticle deposition (difference of reflectance, %). PROC REG and stepwise-backward elimination procedure was performed using SAS (v 9.4) and significance of $P \geq 0.05$ was utilized. Our results indicate that egg production, mass, shell quality, or weight did not affect the cuticle deposition ($P \geq 0.274$) and was positively and linearly dependent on hen age through wk 50 ($P < 0.001$). None of these characteristics including hen age influenced hatchability of fertile eggs ($P \geq 0.161$). However, as eggshell cuticle deposition values increased, percentage of hatch of fertile eggs linearly improved ($P = 0.011$). Embryo diagnosis relationship with eggshell cuticle indicated that neither early nor mid mortalities were influenced by cuticle ($P \geq 0.347$), although higher cuticle deposition contributed to lower late embryonic mortalities ($P = 0.028$). Our results indicate that cuticle is highly dependent on hen age, and possibly plays an important role in improving hatchability of fertile eggs by reducing the number of late embryonic mortalities. Future research is needed to understand nutritional or management strategies that could promote eggshell cuticle deposition in eggs, and the mechanism of how this barrier preserves inner breeder egg quality during incubation.

Key Words: eggshell cuticle, egg mass, late embryonic mortality, hatch of fertile, broiler breeder hen

277 Cryopreservation of rooster semen using different levels of acetamide. Shaimaa K. Hamad*^{GS 1}, Ahmed M. Elomda², Gamal M. Mehaisen¹, Farid K. Stino¹; ¹Department of Animal Production, College of Agriculture, Cairo University, Giza, Egypt, ²Department of Biotechnology, Animal Production Research Institute, Giza, Egypt.

Semen cryopreservation is an important tool in the preservation of animal genetic resources especially with the at-risk and valued species. However, semen cryopreservation is not widely used with the avian species

on the commercial scale. Rooster spermatozoa has particular characteristics compared to that in the other species, such as the smaller contents of cytoplasm, antioxidants and mitochondria, and the higher levels of polyunsaturated fatty acids in the plasma membrane. Therefore, rooster spermatozoa are more susceptible to damage during the cryopreservation process. Thus, the current study was conducted to investigate the impact of different cryopreservation protocols, with acetamide, on sperm motility traits in chicken roosters. Twelve adult chicken roosters were used for semen collection twice a week and the collections were repeated 6 times. The samples were evaluated individually using Computer-Assisted Sperm Analysis (CASA). Then, the samples with more than 60% progressive motility were pooled and the average concentration was 3.9×10^6 sperm/ μ L. The pooled sample was diluted (1:2 v/v) with EK semen extender supplemented with 3%, 6% and 9% Dimethyl acetamide (DMA), and subjected to two different freezing methods (ultra-rapid freezing in pellets and slow freezing in straws). Data were analyzed using Two-way ANOVA, and the means were separated using multiple-wise Duncan test with significance level of $P < 0.05$. Preliminary results indicate that the total motility (TM) and progressive motility (PRG) percentages were higher ($P < 0.05$) in the slow freezing protocol in straws than in the ultra-rapid freezing protocol in pellets (54.7 vs. 47.8% TM and 27.6 vs. 21.3% PRG, respectively). There was no significant effect of DMA levels on the TM (averaged 52.1%) while the PRG motility was significantly ($P < 0.05$) higher in the DMA at 6% (28.0%) than the other levels (24.7 and 23.0% for 3 and 9% DMA, respectively). There was an interaction effect for freezing protocol \times DMA levels on the TM and PRG. The best results were obtained for the TM and PRG in the slow freezing with 6% DMA (60.3 and 33.2%, respectively), followed by the ultra-rapid freezing with 3% DMA (54.3 and 24.1%, respectively). It can be concluded that the slow freezing in straws with 6% DMA maintain sperm motility characteristics after thawing than the ultra-rapid freezing in pellets.

Key Words: Cryopreservation, Roosters, Semen, Dimethyl acetamide, Sperm motility

278 Impact of growth trajectory on sexual maturation in layer chickens. Mohammad A. Bahry*^{GS}, Charlene Hanlon, Sierra Schaus, Gregoy Y. Bedecarrats; Animal Biosciences, University of Guelph, Guelph, Ontario, Canada.

While domestic chickens are seasonal breeders, recent studies in our lab showed that apart from photostimulation, metabolic triggers may independently activate sexual maturation and egg production when a threshold is met. However, the origin, mode of action, and specific target(s) of this metabolic control, and whether it is strain dependent, remain unknown. Rather than body weight (BW), we hypothesize that body composition (BC) and associated specific metabolic signals are involved. Thus, this study was

conducted to determine the BW and BC thresholds triggering spontaneous sexual maturation in 2 strains of layers under differing growth trajectories. Day old Lohman LSL lite (W) and Lohman brown (Br) layer chicks (n=210 each) were raised in brooding cages (n=15; 14 chicks/cage) under ad libitum (AL) feeding, with lighting schedule as per breeder's recommendation. At 8 weeks of age (woa), pullets were randomly allocated into individual cages across two rooms (n=210 cages/room) and assigned to one of 3 experimental growth profiles, including AL, breeder's target (T), restricted 20% below target (R), (n=70 birds/profile/ strain). All hens were maintained on 10 h of light (10 lux) throughout the rest of the study, with no photostimulation. Feed allocation for T and R birds were determined individually on a weekly basis based on actual BWs, with all hens fed the same commercial diet. Blood and tissue samples were collected throughout the study to measure estradiol (E2) levels and organ weights, respectively, and carcasses were subjected to DEXA (Dual-energy X-ray absorptiometry) analyses. All analyses were completed in SAS using the MIXED procedure. Our results show that R treatment slowed ($P < 0.001$) body growth, delayed age at first egg (FE) and egg production ($P < 0.001$), and resulted in lower BW at FE ($P < 0.001$), lower ovary weight and number of follicles ($P < 0.001$), and attenuated plasma E2 levels in both strains. In Br hens, a further delay in the E2 peak was observed. Interestingly, AL feeding resulted in higher growth rate and earlier age at FE ($P < 0.001$) for Br hens only. However, birds fed AL had a higher BW at FE ($P < 0.001$), higher follicle number and ovary weight ($P < 0.001$) in both strains. For DEXA, AL feeding ($P < 0.001$) increased tissue weight, lean weight, and fat deposition while R significantly reduced them ($P < 0.0001$). Of interest, fat deposition remained below 10% in Br hens until 22 woa, time at which a delayed peak in plasma E2 was observed. In conclusion, feed allocation impacted growth and BC in a strain dependent manner, resulting in differing sexual maturation and egg production. Whether these effects are mediated through hypothalamic input is under investigation.

Key Words: Layers, Body weight, Body composition, Sexual maturation, Egg production

279 Cyclosporin A halts ovarian transplant rejection during the first two weeks post-surgery in the domestic turkey (*Meleagris gallopavo*). George B. Hall*^{GS}¹, Janet Beeler-Marfisi¹, Julie Long², Ben J. Wood^{1,3}, Gregoy Y. Bedecarrats¹; ¹Animal Biosciences, University of Guelph, Guelph, British Columbia, Canada, ²ARS-USDA, Beltsville, Maryland, United States, ³The University of Queensland, Gatton, Queensland, Australia.

Biobanked turkey ovaries collected from recently hatched poult could be revived by transplanting the tissue into a recipient bird; however, studies have shown that ovarian transplants become highly infiltrated by T and B-lymphocytes leading to tissue rejection. Using an immunosuppressant to alter this immune response has not

yet been thoroughly investigated, thus, the goal was to determine if mycophenolate mofetil (MMF), cyclophosphamide (CY), or cyclosporin A (CsA) could stop rejection and permit normal graft development. For this study, 7- to 9-day old donors and 2-day old recipient poult were used. Recipients were assigned to 1 of 9 treatment groups, with 3 to 6 poult per group. Treatment groups included: (1) no immunosuppressant 'control'; (2-3) MMF at 100 or 150 mg/kg/day via crop gavage; (4) MMF at 100 mg/kg/day via subcutaneous injection; (5) CY at 200 mg/kg the day before surgery only; (6) CsA at 50 mg/kg/day beginning the day before surgery; (7-9) CsA at 12.5, 25 or 50 mg/kg/day starting the day after surgery. At 14 days post-surgery, blood was collected for white blood cell count (WBC) and recipients were euthanized. Their bursa and thymus were weighed, and ovarian transplants were fixed for analysis. Fixed ovaries were sectioned, stained (H&E), and graded based on appearance (normal or infiltrated by lymphocytes). A one-way ANOVA, followed by a Tukey post-hoc test was used to evaluate differences between treatment groups for WBC, and organ relative weight (RW). Graft appearance was analyzed using a generalized linear model, followed by a pair-wise comparison. Birds receiving CsA had the lowest number of circulating lymphocytes and total leukocytes compared to the controls ($P \leq 0.05$); whereas the number of lymphocytes and total leukocytes were similar between MMF- and CY-treated birds and controls. Birds treated with CY had the smallest ($P \leq 0.001$) bursal RW (0.046 ± 0.004 %), compared to all other treatments. In the controls, 4/5 grafts were graded as being infiltrated with lymphocytes. For the MMF, CY groups, and the CsA 12.5 mg group all transplants were infiltrated by lymphocytes. Grafts in the CsA 25 and 50 mg groups did not show any sign of lymphocyte infiltration ($P \leq 0.001$). Results show that MMF, the most common immunosuppressant used in ovarian transplantation for poultry, did not reduce circulating lymphocytes or transplant infiltration. Although CY significantly decreased bursal RW, it did not stop graft rejection. CsA at the higher doses reduced the number of circulating lymphocytes and prevented lymphocytic graft infiltration. Therefore, CsA at 25 mg/kg/day would be recommended to prevent turkey ovarian graft rejection during the first 2 weeks post-surgery.

Key Words: Ovary, Transplantation, Immunosuppressant, Turkey, Biobanking

280 Impacts of intensive genetic selection on expression of genes regulating calcium and phosphorus uptake and retention in broilers during embryonic and early post-hatch development. Manuel A. Arango*^{GS}, Brett Marshall, Min Lee, Laura Ellestad; Poultry Science, University of Georgia, Athens, Georgia, United States.

Genetic selection of commercial broilers has historically been primarily focused on production parameters, including increased growth efficiency and meat yields. Such selection has subsequently contributed to higher incidence of leg disorders, many of which are a result of skeletal

abnormalities. Bone is comprised of hydroxyapatite, a complex of calcium (Ca) and phosphorus (P) that begins its formation during embryonic ages. This mineralization process is regulated by jejunum absorption and kidney resorption of these minerals. This study sought to define gene expression of Ca and P transporters and chaperones that may have been affected by commercial genetic selection. To this end, total RNA was extracted from kidney and jejunum collected on embryonic days (E) 14, 16, 18, 20 and post-hatch days (D) 0, 1, 3, 5, 7, 10, and 13 from male Cobb 500 and Athens Canadian Random Bred (ACRB) birds, the latter of which are representative of broilers from the mid-1950s ($n=6$). Levels of mRNA for Ca and P transporters and chaperones were measured by RT-qPCR, and target gene expression was normalized to glyceraldehyde 3-phosphate dehydrogenase in jejunum and 18S in kidney. Data were analyzed by 2-way ANOVA followed by the test of least significant difference when ANOVA indicated significance ($P \leq 0.05$). Plasma membrane Ca-transporting ATPase 1 mRNA levels, which moves Ca to interstitial space to be collected by blood vessels, was higher in ACRB kidney at earlier post-hatch ages (D1, $P \leq 0.05$) and higher in Cobb kidney at later ages (D13, $P \leq 0.05$). Expression of Ca sensing receptor (CASR) in kidney, which detects changes in Ca concentration in the distal convoluted tubule, was more highly expressed earlier in Cobb (D0, $P \leq 0.05$) than in ACRB, which had higher expression on later ages (D13, $P \leq 0.05$). In jejunum, CASR expression was higher in Cobb at most post-hatch time points (D1, D3, D7, D13; $P \leq 0.05$). Kidney expression of calbindin (CALB1-28k), a cytoplasmic Ca chaperone, was more highly expressed in Cobb at early embryonic ages (E16, $P \leq 0.05$) and higher in ACRB closer to hatch (E20, $P \leq 0.05$). In contrast to calcium transporters, inorganic phosphate transporter 1 (PiT1) showed higher expression in ACRB at embryonic ages (E20, $P \leq 0.05$), while expression in Cobb was higher at post-hatch ages (D13, $P \leq 0.05$). Furthermore, PiT1 expression in jejunum was higher at all post-hatch days in ACRB ($P \leq 0.05$). These results indicate that genetic selection may have altered Ca and P exchange in modern broilers, which could alter availability of these ions for bone formation at critical ages, therefore leading to reduced leg health in these birds.

Key Words: Genetic selection, calcium regulation, phosphorus regulation, ion transporters, bone health

281 Tissue distribution of genes involved in calcium and phosphorus utilization in the laying hen intestinal tract. Micaela Pinto*^{GS}², Camille Evans¹, Lyssa R. Blair¹, Laura Ellestad¹; ¹Poultry Science, University of Georgia, Athens, Georgia, United States, ²Poultry Science, University of Georgia, Athens, Georgia, United States.

Eggshell calcification requires stringent regulation of calcium (Ca) and phosphorus (P) absorption from the intestinal tract, yet the mechanisms and timing behind Ca and P uptake in laying hens are poorly understood. This study investigated the expression of genes involved in the

transport and regulation of Ca and P in the duodenum, jejunum, ileum, and ceca of mature laying hens. To this end, laying hens containing an actively calcifying egg in the shell gland at 21 weeks of age (n=7) were euthanized, and mucosal scrapings of each section of the gastrointestinal tract were collected. Total RNA was extracted and reverse transcribed using Qiazol and M-MuLV reverse transcriptase, respectively. Levels of mRNA were determined using real-time quantitative PCR, and target gene expression was normalized to glyceraldehyde-3-phosphate dehydrogenase in each sample. Data were analyzed by one-way ANOVA, and means were compared using Tukey's Honest Significant Difference test when ANOVA indicated statistical significance ($P \leq 0.05$). Levels of calbindin 1 (CALB1) were similar throughout the small intestine; however, its expression was significantly lower in the ceca ($P \leq 0.05$). Ca transporter (ATP2B1) was uniformly expressed. Calcium sensing receptor (CASR) and parathyroid hormone receptors 1 and 3 (PTH1R, PTH3R) increased progressively from duodenum to ileum, with expression levels in ileum being significantly higher than in duodenum ($P \leq 0.05$). Inorganic phosphorus transporter 1 (PiT-1) showed the highest levels of expression in the ceca ($P \leq 0.05$), and low levels of expression in the duodenum, jejunum and ileum. Phosphorus transporter 2 (PiT-2), however, increased in expression from the duodenum to the ileum ($P \leq 0.05$), with the cecal expression decreasing from that of the ileum. Calcium uptake may also be altered by vitamin D. Vitamin D receptor (VDR) expression was evenly distributed between small intestinal segments but substantially lower in the ceca ($P \leq 0.05$). Retinoid X receptor alpha increased in expression from the duodenum to the ceca ($P \leq 0.05$), and retinoid X receptor gamma increased from the duodenum to the ileum, with a lower cecal expression of this gene ($P \leq 0.05$). These results indicate that expression of Ca and P related genes generally increased towards the distal parts of the small intestine and ceca, with most genes being expressed at highest levels in the ileum. Given the pattern of Pit-1 and Pit-2 expression, the ceca may play an important role in mediating phosphorus uptake. Taken together, these results suggest that the distal components of the gastrointestinal tract of laying hens may play a larger role than previously thought in mediating Ca and P uptake and utilization during eggshell calcification.

Key Words: Calcium, Phosphorus, Eggshell calcification, Laying hen, Vitamin D

282 Determining the phenotypic shape variation of the premaxillary and dentary bones that may underlie beak shape in two pure layer lines. Sarah Struthers*^{GS 1, 2}, Björn Andersson³, Matthias Schmutz³, Heather McCormack¹, Peter Wilson¹, Ian Dunn¹, Vicky Sandilands², Jeffrey Schoenebeck¹; ¹Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom, ²Scotland's Rural College, Edinburgh, United Kingdom, ³Lohmann Breeders, Cuxhaven, 27472, Germany.

Severe feather pecking (SFP) is a behavior that is often observed in commercial laying hen flocks and has serious consequences for bird welfare as it can result in wounds, cannibalism, and mortality. Beak treatment is an ineffective method of reducing the damage inflicted by SFP but there is significant pressure to eliminate these treatments and rely solely on alternative strategies. In response to this, there has been an increased focus on selective breeding against SFP, but the incorporation of meaningful behavior data into a breeding program can be challenging. Therefore, quantifiable outcomes such as plumage cover, liveability, and beak shape are measured. Substantial natural variation in beak shape exists within non-beak treated layer flocks and beak shape appears to be heritable. There is the potential to use this pre-existing variation and genetically select for hens whose beak shapes are less apt to cause damage during SFP. In order to do this, we must first understand the range of phenotypes that exist for both the external beak shape and the bones that provide its structure. The objective of this study was to determine the variation in premaxillary and dentary bone morphology that exists in two non-beak treated pure White Leghorn layer lines using geometric morphometrics to analyse radiographs. Left lateral head radiographs were taken of 825 hens and the premaxillary and dentary bones were landmarked using ImageJ software. Landmark coordinates were standardised by Procrustes superimposition and the covariation was analysed by principal components analysis and multivariate regression using Geomorph (an R package). For the premaxillary bone, three principal components (PCs) explained 85% of the total shape variation and showed that premaxillary bone shape ranged from long and narrow with pointed bone tips to short and wide with more curved tips. The two lines overlapped significantly in morphospace (shape space); however, they were partially separated by PC1, which described the bone width and angle of curvature. For the dentary bone, two PCs explained 81% of the total shape variation. PC1 described the dentary bone length and width and PC2 explained the angle between the bone tip and its articular process. For both bones, shape was significantly associated with bone size and differed significantly between the two lines. Bone size accounted for approximately 42% of total shape variation for both bones. Together, the results showed a range of phenotypic variation in premaxillary and dentary bone shape. These bone phenotypes will guide further quantitative genetic and behavioral analyses that will help identify which beak shapes cause the least damage when birds engage in SFP.

Key Words: beak shape, bone shape, Lohmann, laying hen, geometric morphometrics

283 Delayed post-hatch feeding alters intestinal stem cell activities and intestinal permeability in broiler chickens. Liang-en Yu*^{GS 1}, Sofia Bialkowski¹, Lydia Schlitzkus¹, Yihang Li²; ¹Animal and Food Science, University of Delaware, Newark, Delaware, United States, ²Animal and Food Sciences, University of Delaware, Newark, Delaware, United States.

Early life adversity alters the developmental trajectory of intestine, leading to functional disorders of nutrient absorption, barrier integrity, and intestinal immunity later in life. Intestinal epithelial stem cells (IESCs) govern the development and homeostasis of intestinal epithelium, which may play important roles in intestinal dysfunction and post-stress recovery. However, the effect of early life stress on the intestinal function and IESCs behavior in poultry had not been fully evaluated. The objective of this study was to evaluate how post-hatch feed deprivation impacts intestinal permeability and IESCs characterizations in broilers. A total of 160 Ross 308 eggs were hatched and allotted into either delayed feeding group (DF, no feed until 72hr post-hatching) or normal feeding group (NF, immediate access to feed). Both NF and DF were housed in the same environment. At d3, d4, d14, and d21 post hatching, ileal samples were collected post 24h-BrdU i.p. injection. Tissues were fixed for morphological analysis and BrdU immunohistochemistry. Ileal mucosa were mounted on the Ussing chamber for FD4 permeability assessment. Crypt cells were isolated for culturing intestinal organoids. Ileal cell proliferation and epithelial differentiation marker genes were quantified via RT-PCR. Student's t-test was performed using GraphPad and significance denoted at $P \leq 0.05$. Our results showed that DF significantly decreased ($P < 0.05$) villus height and crypt depth at d3, d14, but not at d21. However, ileal FD4 permeability was not different at d3, but was significantly higher ($P < 0.05$) in DF birds at d14 and d21 compared with NF control. In vivo proliferation of epithelial cells, indicated by BrdU positive cells, was suppressed in DF group. In vitro activities of IESCs, indicated by enteroids formation rate, size, and expansion index, were significantly reduced ($P < 0.05$) in the DF birds at d3 and d14 post-hatch in comparison with control. After 24h refeeding, the proliferation ability of IESCs from d4 chickens was restored ~50% ($P < 0.05$) compared with control. Interestingly, the expression of IESCs marker gene *Olfm4* was significantly reduced at d3, but numerically upregulated at d21. Epithelial goblet cell marker (*MUC2*) was upregulated ($P = 0.07$) while Paneth cell marker (*LYZ*) were downregulated ($P = 0.09$) in DF bird at d21. Together, the results indicated that early life stress had long term effects on intestinal barrier function, which may be regulated by dynamic IESCs proliferation and differentiation activities. Understanding IESCs regulation during early life development will shed lights on identifying novel strategies for optimal gut health and regeneration under stress.

Key Words: Broilers, Delayed feeding, Enteroids, Intestinal barrier, Gut health

284 Heat stress affects proliferation and differentiation through the mTOR/S6K pathway in breast muscle satellite cells from turkeys with different growth rates. Jiahui Xu*^{GS 1}, Gale M. Strasburg², Kent M. Reed³, Sandra Velleman¹; ¹Animal Sciences, Ohio State University, Wooster, Ohio, United States, ²Department of Food Science and Human Nutrition, Michigan State Poultry. Sci. 100 (E-Suppl 1)

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Newly hatched poult have an immature thermal regulatory system and as homotherms have difficulty handling thermal stress. As muscle stem cells, satellite cells (SCs) are responsible for post-hatch muscle growth by hypertrophy and are sensitive to temperature during the first week after hatch. Heat stress increases both proliferation and differentiation rates of 1-wk-old turkey pectoralis major (p. major) muscle SCs (pmSCs), and growth-selection amplifies the temperature effect. The mechanistic target of rapamycin (mTOR) signaling pathway, which is highly sensitive to heat stress during turkey pmSC differentiation, regulates SC proliferation and myofiber hypertrophy through a downstream effector, S6 kinase (S6K). Thus, it was hypothesized that heat stress will affect mTOR/S6K signal transduction, altering the proliferation and differentiation of pmSCs in a growth-dependent manner. The SCs were isolated from the p. major muscle of 1-wk-old faster-growing modern commercial turkeys (NC) and slower-growing Randombred control line 2 (RBC2) turkeys representing commercial turkeys of 1966. Satellite cells were incubated at 38 or 43°C during proliferation and differentiation. Protein expression was determined by western-blot analysis, and phosphorylated mTOR and S6K were normalized to their total expression. Small interference RNA targeting mTOR was transfected into the SCs. After transfection, proliferation, differentiation, and gene expression were determined by DNA concentration, creatine kinase assay, and real-time quantitative polymerase chain reaction, respectively. Data were analyzed by MIXED procedure in SAS with $P < 0.05$ considered significant. During proliferation and differentiation, both mTOR and S6K showed higher phosphorylation levels ($P < 0.01$) in the NC line compared to the RBC2 line at 38 and 43°C. Phosphorylation of both mTOR and S6K increased in both lines ($P < 0.01$) at 43°C compared to 38°C, and a greater increase was observed in the RBC2 line. Knocking down mTOR expression decreased ($P < 0.01$) proliferation and differentiation in both lines at 38 and 43°C with the RBC2 line showing a greater linear reduction than the NC line. Expressions of myoblast determination protein 1 and myogenin during proliferation and differentiation were decreased in both lines ($P < 0.01$) after knocking down mTOR at 38 and 43°C. In the RBC2 line, a greater reduction in both genes was observed during proliferation. These data suggest heat stress increases the breast muscle growth potential partially through mTOR/S6K pathway, and pmSCs from the modern commercial turkeys are less dependent on mTOR-mediated growth with the potential to affect heat stress-induced increases in breast muscle growth and yield.

Key Words: differentiation, heat stress, mTOR, proliferation, satellite cell

285 Effect of cell culture plate gelatin coating method on density of primary broiler chicken skeletal muscle satellite cells. Brittany L. Wall*^{GS}, Caroline R. Gregg, Joshua J. Flees, Jessica D. Starkey; Poultry Science, Auburn University, Auburn, Alabama, United States.

The hypertrophic growth of skeletal muscle is dependent on the function of myogenic stem cells called satellite cells (SC). Protocols for the *in vitro* culture of primary broiler chicken SC are not standardized in the literature. Therefore, the objective was to determine the impact of gelatin plate coating method on SC density after 72 h in culture. Six 2-cm² wells on 3 replicate tissue culture plates were coated with 0.1% gelatin diluted in sterile Ultrapure water using 1 of 2 methods. Half of the wells (n = 18) were coated with 500 µL of 0.1% gelatin per well, incubated at room temperature (RT) for 30 min, liquid gelatin was aspirated off, and wells were dried at RT for 30 min prior to SC plating (DRY method). The other half of the wells (n = 18) were coated with 500 µL of 0.1% gelatin per well, incubated at RT for 30 min, liquid gelatin was aspirated off, and SC were immediately plated on the wet gelatin (WET method). Primary SC isolated from *Pectoralis major* muscles of 24, male, 15-d-old broilers were pooled and plated in Low Glucose Dulbecco's Modified Eagle's Media supplemented with 10% chicken serum, 5% horse serum, 1% antibiotic/antimycotic, and 0.1% gentamicin. At 24 h post-plating, the 3 replicate plates were rinsed, and fed fresh media. At 72 h post-plating, plates were fixed, immunofluorescence stained to detect SC expressing the commonly used SC marker, paired box 7 (Pax7), and nuclear counterstained with DAPI. Plates were imaged using a Nikon Ti-U Eclipse® inverted fluorescence microscopy system and Nikon Elements software. Five digital images per well were captured and populations of DAPI+:Pax7+ cells were enumerated, and their density expressed on a cells per mm² basis. Data were analyzed using the GLIMMIX procedure of SAS (V9.4) with gelatin coating method as the fixed effect and culture well as the experimental unit. Multiple comparisons of least squares means were performed with the PDIF option at $P \leq 0.05$. As hypothesized, gelatin coating method did not alter the density of Pax7+ SC (DRY = 41 per mm²; WET = 40 per mm² ± 3; $P = 0.7315$) at 72 h post-plating. A major advantage of the DRY method is that the method allows for greater flexibility in the timing of SC plating without appearing to compromise SC proliferation. These data suggest that either the DRY or WET gelatin plate coating method can be successfully utilized for culturing *Pectoralis major* primary SC from broilers.

Key Words: Skeletal muscle satellite cell, broiler chicken, gelatin plate coating method, satellite cell culture, muscle stem cell

286 Effect of *in vitro* culture temperature on heterogeneity of myogenic regulatory factor expression in primary broiler chicken satellite cells. Caroline R. Gregg*^{GS}, Joshua J. Flees, Brittany L. Wall, Jessica D.

Starkey; Poultry Science, Auburn University, Auburn, Alabama, United States.

Satellite cells (SC) mediate post-hatch skeletal muscle growth and repair by proliferating and fusing with existing fibers to increase myonuclear DNA content which increases myofibrillar protein synthesis potential. Culture conditions for broiler SC are not standardized in the literature. Broiler body temperature ranges from 40 to 42 °C. The objective of this study was to determine the optimal incubation temperature for culturing primary SC isolated from the pooled *Pectoralis major* (PM) muscles of 24, male, 15-d-old broilers. Primary SC were plated in triplicate on 15.6-mm, gelatin-coated wells on 3 parallel tissue culture plates. They were cultured in low glucose Dulbecco's Modified Eagle's Medium supplemented with 10% chicken serum, 5% horse serum, 1% antibiotic/antimycotic, and 0.1% gentamicin. Cultures were incubated at 40, 41, or 42 °C for 96 h (n = 3 plates with 3 wells per temperature). At 24 and 72 h post-plating, wells were rinsed with serum-free media, and fresh media was added. At 96 h post-plating, cultures were stained using indirect immunofluorescence with a DAPI nuclear counterstain to detect SC expressing the common SC markers and myogenic regulatory factors (MRF), paired box 7 (Pax7) and myogenic differentiation factor 1 (MyoD). All wells (n = 27) were imaged using a Nikon Ti-U Eclipse® inverted fluorescence microscopy system with Nikon Elements software. Images of 5 random fields were captured per well, and populations of DAPI+ only, DAPI+:Pax7+, DAPI+:MyoD+, and DAPI+:Pax7+:MyoD+ cell populations were enumerated and expressed on a mm² basis. Total MRF- or non-myogenic (DAPI+:Pax7-:MyoD-), total MRF+ or myogenic (DAPI+:Pax7+, DAPI+:MyoD+, DAPI+:Pax7+:MyoD+), and total cells (DAPI+) were calculated from the cell taxonomy data. Cell populations were expressed as a proportion of total DAPI+ cells. Data were analyzed using the GLIMMIX procedure of SAS (V9.4) with temperature as the fixed effect and well as the experimental unit. Least squares means were separated using the PDIF option at $P \leq 0.05$. Cultures incubated at 40 °C had more total DAPI+ ($P < 0.0001$), MyoD+ ($P < 0.0001$), Pax7+:MyoD+ ($P < 0.0001$), and total MRF+ ($P < 0.0001$) cells compared with those incubated at 41 °C and 42 °C. The density of both DAPI+:Pax7+ ($P = 0.0212$) and total MRF-, non-myogenic ($P = 0.0071$) cells was greatest in SC cultured at 42 °C. The 40 and 41 °C incubation temperatures produced higher proportions of total MRF+ cells compared with those cultured at 42 °C. Culturing PM broiler primary SC at 40 °C appears to best promote proliferation. Further investigation of SC doubling time, differentiation, and myotube fusion when cultured at chicken body temperature is warranted.

Key Words: skeletal muscle satellite cell, broiler chicken, cell culture temperature, myogenic regulatory factor expression, proliferation

287 Effect of basal plating culture media on primary broiler chicken muscle satellite cell myogenic regulatory

factor expression heterogeneity. Joshua J. Flees*^{GS}, Caroline R. Gregg, Brittany L. Wall, Jessica D. Starkey; Poultry Science, Auburn University, Auburn, Alabama, United States.

Broiler chicken satellite cells (SC) isolated from *Pectoralis major* (PM) muscles provide a useful *in vitro* model to study broiler muscle growth as they play an essential role in post-hatch muscle growth and repair. There is little consistency in the literature regarding optimal conditions for promoting sufficient proliferation of SC and their differentiation into nascent myofibers called myotubes in culture. Therefore, the objective of this study was to determine the effect of different basal plating media on the heterogeneity of myogenic regulatory factors (MRF) expression in SC. Three parallel plates of SC isolated from pooled PM muscle from 12 (19 d-old) male broiler chicks were plated on triplicate wells in 1 of 3 basal media: McCoy's 5A (MCCOY), high glucose Dulbecco's Modified Eagle's medium (HGDMEM), or low glucose DMEM (LGDMEM). All basal media were supplemented with 10% chicken serum, 5% horse serum, 1% antibiotic/antimycotic, and 0.1% gentamicin. SC were cultured at 40 °C with 18% O₂ and 5% CO₂ on 9 wells (n = 3 per basal media) on 24-well culture plates coated with 0.1% gelatin. Twenty-four h post-plating, all plates were rinsed twice with serum-free LGDMEM and cultured for an additional 48 h in LGDMEM containing 10% chicken serum, 5% horse serum, 1% antibiotic/antimycotic, and 0.1% gentamicin. After 48 h in proliferation media (72 h post-plating), cultures were immunofluorescence (IF) stained to detect SC expressing the common SC markers and MRF, paired box 7 (Pax7), myogenic differentiation factor 1 (MyoD), and myogenic factor 5 (Myf-5). After IF staining, a DAPI nuclear stain was applied. Five fields per well were imaged using a Nikon Ti-U Eclipse® inverted fluorescence microscopy system and Nikon Elements software. Cell populations were enumerated DAPI+, Pax7+, MyoD+, Myf-5+, Pax7+:MyoD+, Pax7+:Myf-5+, MyoD+:Myf-5+, and Pax7+:MyoD+:Myf-5+ and densities expressed on a cm² basis. Total MRF- (DAPI+:MRF-) and total MRF+ (DAPI+:MRF+) were calculated. Cell populations were also expressed as a proportion of total DAPI+ cells (TDAPI). Data were analyzed using the GLIMMIX procedure of SAS (V9.4) with well serving as the experimental unit. Multiple least square mean comparisons were performed using the PDIF option at $P \leq 0.05$. Cultures plated in HGDMEM basal media tended to have smaller PAX7+ SC populations at 72-h post-plating compared with SC plated in MCCOY ($P = 0.0965$). Interestingly, basal plating culture media did not alter MyoD+, Myf-5+, Pax7:MyoD+, Pax7+:Myf-5+, MyoD+:Myf-5+, Pax7+:MyoD+:Myf-5+, or total MRF+ SC populations ($P = 0.1594$). Overall, these results indicate that plating primary SC cultures in any of the 3 basal medias tested produce similar populations of double MRF+ SC.

Key Words: skeletal muscle satellite cell, broiler chicken, cell culture, plating media, myogenic regulatory factor

heterogeneity

288 Temporal and tissue-specific mRNA expression of glucose and amino acid transporters in broilers. Shailes Bhattarai*^{GS}, Ana Villegas, Todd Applegate, Laura Ellestad; Poultry Science, University of Georgia, Athens, Georgia, United States.

In poultry, nutrient absorption is facilitated by the gastrointestinal tract, and nutrients are transported to liver and muscle for further metabolic processing and partitioning. Glucose is a key energy substrate, while amino acids (AA) are critical for protein accretion. In mammals, age and tissue-specific physiological mechanisms regulating nutrient uptake and utilization are well-studied. However, such information is lacking in broilers, a commercially important species for which understanding these processes might enhance production efficiency. We investigated expression of glucose transporters (GLUT1, GLUT2, GLUT8, and GLUT12) and AA transporters (CAT1, CAT2, LAT1, and yLAT2) in the jejunal mucosa, liver, and breast muscle (*Pectoralis major*) of male Ross broilers on post-hatch day (D) 17 and D28 (n=12 replicates per age). Expression of mRNA for glucose and AA transporters was measured by RT-qPCR. Data were analyzed using ANOVA followed by Fisher's Least Significant Difference test, and differences were considered significant at $p \leq 0.05$. In jejunal mucosa, mRNA expression of GLUT1 significantly decreased between D17 and D28, while expression of GLUT2 increased significantly during this time. Expression of GLUT1 and GLUT8 mRNA increased significantly on D28 in liver, while mRNA expression of GLUT8 and GLUT12 were downregulated on D28 in breast muscle. Both in jejunal mucosa and liver, CAT2 and yLAT2 were elevated significantly on D28. In contrast, breast muscle mRNA expression of CAT1, CAT2, and LAT1 were downregulated on D28, while yLAT2 was upregulated at this age. Increased expression of select insulin-responsive (GLUT2, GLUT8, GLUT12) glucose transporters in jejunal mucosa and liver and a concomitant decrease in breast muscle suggests that, in older broilers, these tissues differ in their sensitivity to circulating insulin and that basal uptake of glucose by breast muscle is adequate to support the rapid growth of this tissue at this age. Further, since GLUT8 and GLUT12 are bidirectional glucose transporters, the decrease in their expression in breast muscle on D28 potentially limits glucose movement out of this tissue to further support the metabolic process of muscle accretion. CAT2 and yLAT2 transport the essential AA arginine and lysine, and their increased expression in jejunum and liver on D28 would support the increased requirement for these AA as the birds grow. Reduced expression of CAT1, CAT2, and LAT1 on D28 in breast muscle is likely reflective of reduced protein turnover in this tissue, again contributing to rapid muscle accretion at this age. Together, these results provide insights into understanding how nutrients are partitioned during different phases of juvenile broiler growth.

Key Words: Jejunum, liver, nutrient partitioning, Pectoralis major, transporters

289 Diminished insulin-like growth factor binding protein expression in the liver and muscle of peri-hatch broilers is associated with the transition from embryogenesis to juvenile development. Lauren A. Vaccaro*^{GS}, Kyle Herring, Abigail M. Wilson, Emma England, Laura Ellestad; Poultry Science, University of Georgia, Athens, Georgia, United States.

Insulin-like growth factor (IGF) 1 and 2 are regulators of vertebrate growth and their activities are modulated by IGF binding proteins (IGFBPs). In chickens, proper growth and development require a coordinated transition to occur between late embryogenesis and early post-hatch. The objective of this study was to determine if expression of genes regulating IGF signaling action changed throughout this developmental transition. Liver and breast muscle (Pectoralis major) were collected from Ross 308 males at embryonic day (e) 12, e14, e16, e18, e20, and post-hatch day (d) 0, d1, d3, d5, d7, d10, d14, and d21. Total RNA was extracted from 6 birds at each age (n=6). Expression of mRNA was determined by reverse-transcription real-time quantitative PCR, and target mRNA levels were normalized to 18S rRNA levels. Data were analyzed by one-way ANOVA and post hoc means comparisons were made with the test of least significant difference when ANOVA indicated significance ($P \leq 0.05$). Age-induced differences in expression were observed in both liver and breast muscle. Liver IGF1 and IGFBP4 increased after hatch despite low embryonic expression ($P \leq 0.05$). On e20, liver expression of IGF1R, IGFBP1, IGFBP2, and IGFBP3 decreased but then increased again by d3 and remain elevated throughout the juvenile period ($P \leq 0.05$), although none returned to embryonic expression levels. Similarly, IGFBP5 and IGFBP7 in liver were diminished between d0 and d3 and rebounded following hatch ($P \leq 0.05$). In contrast to the liver, embryonic IGF1R, IGFBP2, IGFBP4, and IGFBP5 in breast muscle dropped after d1 and remained low until d21 ($P \leq 0.05$). The IGFBPs regulate IGF signaling in part by preventing IGFs from binding to their receptor. Diminished IGFBP expression during the peri-hatch period and reduced juvenile expression, particularly in breast muscle, suggests that enhanced IGF signaling could be necessary to induce rapid post-hatch growth observed in commercial broilers. The reduction of IGFBP mRNA overall might enhance sensitivity of skeletal muscle to both circulating endocrine and local paracrine IGFs, although the strength of this effect could differ across the IGFBPs.

Key Words: insulin-like growth factors, insulin-like growth factor binding proteins, liver, muscle, hormonal signaling

290 Effect of maternal and post-hatch supplementation of 25-hydroxycholecalciferol on *in vivo* broiler chicken duodenal crypt cell proliferation. Samuel F. Leiva*^{GS}¹, Luis P. Avila¹, Gerardo A. Abascal-

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Undifferentiated proliferating crypt cells (stem cells and transit amplifying cells) are the source for intestinal epithelial development and regeneration. Epithelial cell proliferation is necessary during the early post-hatch stage to develop a functional epithelium as the bird transitions to a solid diet. However, it is a metabolically expensive process that diverts energy and amino acids from skeletal muscle accretion. Previous studies have found that post-hatch supplementation of broiler diets with the vitamin D (D3) metabolite, 25-hydroxycholecalciferol (25OHD3), affects the small intestine morphology. A randomized complete block design experiment with a 2×2 factorial treatment arrangement was conducted to assess the effect of combining maternal (MDIET) and post-hatch (PDIET) dietary 25OHD3 inclusion on duodenal (DUO) crypt cell proliferation of young broiler chickens. All diets were formulated to provide 5,000 IU of vitamin D. Broiler breeder hens were offered 1 of 2 MDIET: 5,000 IU D3 per kg of feed (MCTL) or 2,240 IU of D3 + 2,760 IU of 25OHD3 per kg of feed (M25OHD3) from wk 25 to 41. Male broiler offspring (n = 480) hatched from eggs collected during breeder age wk 41 were allotted in raised floor pens (4 birds per pen from d 0 to 7 and 1 bird per pen from d 8 to 21). Chicks were fed 1 of 2 PDIET (starter d 0 to 21): 5,000 IU D3 per kg of feed (PCTL) or 2,240 IU D3 + 2,760 IU 25OHD3 (P25OHD3). DUO samples (n = 12 birds per treatment per day) were collected on d 3, 6, 9, 12, 15, 18 and 21 for cryohistological and immunofluorescence analysis to facilitate the enumeration of total cell nuclei (DAPI+) and mitotically active cells (BrdU+) and calculate the proportion of proliferating cells (PPC) per duodenal crypt. Data were analyzed using SAS (V9.4) PROC GLIMMIX and means were separated at $P \leq 0.05$. Bird age impacted crypt PPC with the greatest PPC per duodenal crypt observed on d 3 and 12 (39.30 and 41.11%, respectively), and the lowest PPC per crypt observed on d 21 (25.32 %; $P < 0.0001$). A MDIET \times PDIET interaction was observed for the PPC per crypt on d 3 where M25OHD3:PCTL broilers had greater PPC in their crypts than MCTL:PCTL birds ($P = 0.002$). No MDIET \times PDIET were observed at any other time points. MDIET altered duodenal crypt cell proliferation on d 6 post-hatch as MCTL broiler offspring had a greater crypt PPC than those from hens fed M25OHD3 (36.71 vs. 33.89 % respectively; $P = 0.0002$). Overall, it appears that maternal supplementation with 25OHD3 may influence early post-hatch small intestinal physiology of broilers. Further research is warranted to understand the underlying mechanisms behind these changes.

Key Words: 25-hydroxycholecalciferol, vitamin D, intestinal development, cell proliferation, duodenum

291 Effects of antibiotic growth promoters on growth performance and intestinal development in commercial broilers. Charles K. Meeks*^{GS}, Laura Ellestad; Poultry Science, University of Georgia, Athens, Georgia, United States.

Subtherapeutic levels of antibiotics have historically been used in the broiler industry for the purpose of growth promotion. Implementation of the Veterinary Feed Directive has barred the continuation of this practice, generating a need to establish antibiotic growth promoter (AGP) alternatives for use in broiler production. However, the mechanism by which AGPs influence broiler growth efficiency remains unknown, complicating the search for a suitable replacement. The objective of this study was to investigate the effects of AGPs on growth performance and intestinal development. Male broiler chicks (108) were individually caged in an environmentally controlled room between D10 and D36 and received one of two grower (d10-d24) and finisher (d24-d36) diets (n=54/diet): an antibiotic-free control diet (ABF) or a diet containing subtherapeutic levels of bacitracin methylene disalicylate (BMD; 55 mg/kg diet). Birds and feed were weighed on d10, d24, and d35 for determination of individual body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR; g BWG/g FI). On d36, the 4 birds with the lowest (high efficiency; HE) or highest (low efficiency; LE) FCR from each diet were used for sample collection (n=4), resulting in 4 groups: ABF-HE, ABF-LE, BMD-HE, and BMD-LE. Body weight and intestinal tract weights were determined, and mucosal

scrapings from jejunum and ileum, excreta, and the right ceca were collected. Data were analyzed by 2-way ANOVA, and post hoc means comparisons were made with the test of least significant difference when ANOVA indicated statistical significance (P<0.05). Birds fed the BMD diet (BW=2656.3 g, BWG=2367.9 g) were significantly heavier on D35 and gained significantly more weight between D10 and D35 than birds fed the ABF diet (BW=2583.5 g, BWG=2292.96 g) (n=54). Among the sampled birds (n=4), there was a significant diet*group interaction for FCR, with the ABF-HE (1.35) and BMD-HE (1.36) FCRs being significantly lower than the two LE groups but not different from each other. FCR for the BMD-LE group (1.55) was significantly lower than that of the ABF-LE group (1.65). There was a main FCR effect on % excreta dry matter, which was significantly higher in LE birds at 26% compared to HE birds at 21%. There were no interaction or main diet effects for intestinal tract weights, but there was main FCR effect where the jejunum (% BW) of LE birds was heavier than that of HE birds. Total aerobic plate counts in jejunum, ileum, and ceca were not different between groups. These data suggest that while AGPs did significantly improve FCR in LE birds and BWG, direct effects on the intestinal tract size or aerobic microbial population are not responsible for this improvement.

Key Words: BMD, feed conversion ratio, intestine, broiler, growth efficiency

Processing and Products

292 Immersion chiller media characterization and effects on peracetic acid half-life rates. Daniel Sabo*, Stephanie Richter; Georgia Tech Research Institute, Atlanta, Georgia, United States.

The poultry industry relies on peracetic acid (PAA) as an antimicrobial agent in an effort to control microbial contamination. This reliance does not equate to an understanding of PAA stability and decay within immersion chillers. There are a growing number of anecdotal reports indicating PAA's rapid decay in the presence of organic carbon that are in the forms of total suspended solids (TSS), total dissolved solids (TDS), fats, oils, and greases (FOGs). In an effort to inform industry of how individual components of organic carbon impact PAA's stability, we first must understand how these parameters change throughout a processing day. This project characterized chiller water samples from both pre-chiller and main chiller from first WOG (whole bird without giblets) in until last WOG out at two different plants. All samples were analyzed for pH, PAA, TSS, TDS, and FOG concentration to aid in later PAA stability studies. A third plant characterization is planned and statistical analysis of all three plants will be performed once all data is collected. For the first two plants, the concentrations of TSS, TDS and FOG were low in the beginning of chill in both chiller types. The pre-chiller reached "steady state" within 3-4 hours, while the main chiller TSS, TDS, and FOG continually increased throughout the day. Utilizing this data, organic carbon components were individually tested for their effects on PAA stability. All tests began with PAA at an initial 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. Half-lives were calculated using an exponential decay formula, while averages and standard deviations were calculated using Excel formulas. PAA in potable water (pH 9.0) acted as a control group for decay rate with a half-life of 145 minutes. TSS at 2000 ppm resulted in a half-life of 80 minutes. TDS at 1750 ppm had a large effect on PAA, with a half-life of 6 minutes. Components that contribute to TDS, including proteins and organic nitrogen, were also tested. Bovine serum albumin (BSA) protein at 1800 ppm resulted in half-life of 141 minutes. Organic nitrogen at 250 ppm had the largest effect on PAA with a half-life of 1 minute. The majority of organic carbon species in the pre-chiller reach a steady state within a few hours. On the other hand, those same species continually increase in the main chiller throughout the processing day. As for the species that has the largest effect on PAA stability, it was shown that TDS and by extension, organic nitrogen cause PAA to rapidly decay.

Key Words: peracetic acid, PAA, PAA decay, characterization, chiller

293 Combination of water immersion chilling and subzero saline chilling improved chilling efficiency and meat tenderness of broiler carcasses. Kota Kawamura*¹, Danyi Ma¹, Anisse Pereira², Ike kang¹; ¹Agriculture, California Polytechnic University - San Luis Obispo, Fullerton, California, United States, ²Food Science, Cal Poly San Luis Obispo, Los Osos, California, United States.

Chilling freshly slaughtered carcasses is vital to ensure meat quality and product wholesomeness. Water immersion chilling (WIC) of poultry carcasses is widely practiced in the United States. However, poor or delayed chilling conditions reduce meat quality and product safety. Recently, a novel chilling technology known as subzero saline chilling (SSC) has demonstrated improvement of chilling efficiency and product safety. The objective of this study was to further investigate the effects of SSC on broiler carcasses in various chilling conditions. The experiment was conducted in two replicates. In each replicate, a total of thirty 6-week-old commercial broilers were obtained and conventionally processed with live weights 2.3 – 3.3 kg. After evisceration, carcasses were immediately chilled by randomly assigning to one of the five solutions: 1) WIC (control) in 0% NaCl/0.5 oC, 2) SSC in 4% NaCl/-2.41 oC, 3) SSC-WIC: SSC in 4% NaCl/-2.41 oC followed by WIC in 0% NaCl/0.5 oC, 4) WWIC-WIC: warm-WIC in 0% NaCl/10.0 oC followed by WIC, and 5) WIC-SSC: WIC followed by SSC. The internal temperature of breast fillets was monitored during chilling. Razor blade shear force (RBSF), sarcomere length, pH, R-value, and instrumental color (L^* , a^* , and b^*), were evaluated in breast fillets. The experiment was a randomized block design. Data were analyzed using the PROC MIXED of SAS, with the replicate and chilling methods as fixed effects and animal ID as random effect. Before chilling, the internal carcass temperature was 40.2oC on average. Temperature was reduced to 4.2 to 5.3oC, with average chilling times of 78.6, 87.1, 87.3, 89.5, and 129.4 min in SSC, SSC-WIC, WIC, WIC-SSC, and WWIC-WIC, respectively. Chilling yield was higher in WIC-SSC (103.18 ± 1.65 %) compared to the other four chilling conditions (101.49 ± 1.01 %, pooled; $P < 0.05$). After cooking, the most tenderized fillets were noticed in SSC (8.46 ± 1.79 N) and WIC-SSC (8.53 ± 1.88 N), which showed 27.4% reduction of RBSF compared to the remaining chilling methods (11.7 ± 2.22 N, pooled; $P < 0.05$). Sarcomere length was longer in the fillets of carcasses that were chilled in WIC-SSC and SSC than in the remaining chilling solutions ($P < 0.05$), supporting the results of fillet tenderness. No statistical significance was observed for breast cooking yield, muscle pH, R-value, or skinless breast color, regardless of chilling method. These results indicate that SSC and WIC-SSC significantly improve carcass chilling efficiency and meat tenderness. Additional research is required to assess bacterial population and salt pickup level on carcasses during chilling as well as shelf-life of the broiler product and recycling of the red water (saline

solution) after chilling.

Key Words: sub-zero saline chilling, water immersion chilling, chilling efficiency, meat tenderness

294 Sub-zero saline chilling improved chilling efficiency and bacterial reduction of turkey carcasses.

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The poultry industry has attempted to improve chilling efficiency, meat quality, and product safety. The purpose of this research was to investigate the effects of sub-zero saline chilling (SSC) on chilling efficiency and microbial safety of tom turkey carcasses. A total of 12 eviscerated turkey carcasses (2 carcasses/treatment for 2 replications; ~ 30 kg/carcass) were randomly picked from the processing line in a local turkey processing plant. They were immediately chilled using one of the three solutions: 1) 0%NaCl/0.5°C (control), 2) 4% NaCl/-2.41°C, and 3) 8% NaCl/-5.08°C. Before chilling, one mid-size tom per treatment was selected, and a thermometer was inserted to the center of breast fillet of the tom to monitor the internal temperature during chilling. After chilling, breast skin (~ 25 g/carcass) was aseptically excised for bacterial enumeration for mesophilic aerobic bacteria (MAB), *Escherichia coli* (*E. coli*), and total coliforms. Data were analyzed using one-way ANOVA and a completely randomized design was used. A post-hoc analysis was performed using Duncan's multiple range test to evaluate difference among treatments at $P < 0.05$. During chilling, carcass temperature was reduced most effectively in 8% NaCl/-5.08 °C (136 min), followed by 4% NaCl/-2.41 °C (175 min) and 0%NaCl/0.5 °C (325 min). After chilling, bacterial populations of MAB, *E. coli*, and total coliforms were significantly reduced in both SSC solutions over the control, except the MAB in 4% NaCl/-2.41 °C. Based on these results, chilling of turkey carcasses in subzero saline solution appears to be a better option than control solution, especially in 4% NaCl/-2.41 °C solution considering the high salt issue in 8% NaCl/-5.08 °C during wastewater treatment. Further research needs to be performed to confirm chilling solution effects on bacterial reduction of turkey carcasses before and after chilling.

Key Words: Sub-zero saline chilling, chilling efficiency, Bacterial reduction, Turkey chilling

295 Temperature stress and poultry meat quality: a meta-analysis. Emily M. Leishman*¹, Jennifer Ellis¹, Nienke van Staaveren¹, Shai Barbut², Ryley Vanderhout¹, Vern Osborne¹, Ben J. Wood^{3, 4, 1}, Alexandra Harlander¹, Christine F. Baes^{1, 5}; ¹Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, ²Food Science, University of Guelph, Guelph, Ontario, Canada, ³School of Veterinary Science, University of Queensland, Gatton, Poult. Sci. 100 (E-Suppl 1)

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Temperature stress has consequences for poultry health and welfare, productivity, and industry profitability by negatively affecting body weight and feed efficiency. Exposure to temperatures outside the thermal comfort zone may also result in meat quality defects, which cost the broiler and turkey industries millions of dollars annually. To determine the effect of temperature stress on poultry meat quality, a meta-analysis was conducted. A systematic literature search was used to identify 48 publications which met the criteria for inclusion. Treatment temperature (°C) and treatment duration were extracted from the chosen studies to define temperature stress. Treatment duration was further categorized for analysis into acute (≤ 24 hours) or chronic (> 24 hours) treatments. The meat quality parameters considered for this study were lightness (L^*), redness (a^*), yellowness (b^*), pH (initial and ultimate), drip loss, cooking loss, and shear force. Linear mixed model analysis, including study as a random effect, was used to determine the effect of temperature and treatment duration on the chosen meat quality traits. Prediction models were evaluated by performing a k-fold cross-validation to estimate test error. Treatment temperature had a significant effect on all studied meat quality traits. As treatment temperature increased, meat colour was lighter, more yellow, and less red. Furthermore, at high temperatures, meat pH was lower, had increased moisture loss, and higher shear force. The interaction between treatment temperature and duration was significant for almost all traits; however, whether acute or chronic stress had a larger impact on meat quality was unclear. Acute stress had a larger effect on ultimate pH, and chronic stress had a larger impact on colour traits (L^* and a^*). The results of this meta-analysis confirm that ambient temperature affects poultry meat quality and quantifies these effects across a large dataset of published studies. However, effects estimated were generally small, and it could be challenged whether they have practical significance from a processing perspective. Further studies should be conducted to better investigate the effect of temperature duration as well as other factors like effective temperature (including humidity, ventilation, etc.) and production traits (i.e., growth rate, feed efficiency, etc.).

Key Words: meta-analysis, meat quality, temperature stress, broiler, turkey

296 Evaluation of toughness and firmness of pet treats developed from broiler chicken carcass frames and wooden breast combinations.

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Pet treats are high-value products that can be developed using various ingredients. To maximize profits without sacrificing positive attributes, quality low-value ingredients can be utilized. Among these ingredients are co-products from broiler chicken processing. Carcass frames (CF) and Wooden Breast (WB) meat are low-priced products that could potentially be transformed into pet treats. To transform these products, a stabilizing agent such as sodium alginate + encapsulated calcium lactate (ALGIN) can be included in the mixture to improve the consistency of the final product. The objective of this study was to assess toughness and firmness of pet treats developed from 5 different CF:WB mixtures with 2 ALGIN inclusions. Severe (score 3) WB and CF were ground using a Thompson 3000 industrial grinder and mixed in the following ratios: 0%CF-100%WB, 25%CF-75%WB, 50%CF-50%WB, 75%CF-25%WB, and 100%CF-0%WB. Mixtures were combined with 1 of 2 ALGIN concentrations (0.500% sodium alginate + 0.425% encapsulated calcium lactate (0.5x), and 1.00% of sodium alginate + 0.85% encapsulated calcium lactate (1x) to produce 10 treatments. Each treatment was extruded into a 52-mm diameter casing, wrapped in plastic wrap, and refrigerated at 1 °C for 16 h and frozen at -11 °C for 3 h prior to being sliced into 3-mm thick slices. Slices were cooked in a 149 °C Koch smokehouse (target internal temperature 74 °C; 6-8 min). Oven temperature was decreased to 66 °C until water activity reached 0.80 (4 h). Ten slices per treatment were analyzed with a shear force test using a TA-42 probe with a 3 mm thick, 70 mm wide and 45° chisel edged blade. This test measured toughness (total force over distance) and firmness (peak force). Data were analyzed using the GLIMMIX procedure of SAS (V9.4) and least square means were separated using the PDIF option at $P \leq 0.05$. An interaction among ALGIN inclusions and CF:WB mixtures for both variables measured was observed. Firmness increased as WB proportion increased, while 0%CF-100%WB and 75%CF-25%WB mixtures with 1x ALGIN were firmer than their 0.5x ALGIN equivalents ($P < 0.0001$). Similarly, higher WB and ALGIN inclusions produced tougher slices, but ALGIN did not impact the 100%CF- 0%WB combination ($P < 0.0001$). Low-value broiler chicken processing co-products, CF and WB were successfully combined with ALGIN to develop pet treats, increasing the value of such co-products.

Key Words: toughness, firmness, carcass frame, pet treat, Wooden Breast meat

297 Differences in carcass yield and breast meat quality parameters in fast- and slow-growing chickens. Jaroslav Valenta*^{GS}, Eva Tumova, Darina Chodova; Department of Animal Science, Czech University of Life Sciences Prague, Prague, Czechia.

In Europe, increased consumer interest in chicken meat from alternative farming systems along with forthcoming legislative changes pose major challenges for the poultry industry. To meet future demand, the use of suitable chicken

genotypes in conventional and organic breeding systems is essential, as is the feeding of cockerels of egg-production poultry. In this study, the carcass yield and differences in breast meat quality of fast- (Ross 308) and slow-growing dual-purpose (ISA Dual) genotypes of chickens were evaluated. 200 cockerels were fed to a final weight of 2 kg under identical conditions. A random selection of 10 birds of each genotype were slaughtered for carcass analysis. Pectoralis major (PM) muscle samples were taken to determine the physical and chemical parameters of the meat quality. Data were analysed by analysis of variance (SAS Institute, Inc., 2013) and significance evaluated using t-test. The Ross 308 reached 2 kg live weight aged 32 days and the ISA Dual aged 74 days. Neither genotype of chicken showed a significant difference in dressing percentage. However, the fast-growing chickens had a significantly higher breast meat percentage ($P \leq 0.001$) and lower abdominal fat content percentage ($P = 0.005$). Regarding the meat's physical characteristics, the pH measured 24 hours post-mortem in PM differed by genotype ($P \leq 0.001$), with lower values in the ISA Dual than the Ross 308. No significant difference was observed in any L*, a*, b* colour values. Higher ($P \leq 0.001$) cooking loss of the Ross 308 indicates a lower water holding capacity than that of the ISA Dual. The chemical composition of the breast meat was determined using the AOAC method (2005). ISA Dual chickens had a significantly higher ($P \leq 0.001$) proportion of dry matter, protein, and ash, while lower ($P \leq 0.001$) fat and cholesterol content than Ross 308 chickens. As expected, due to the longer feeding period, the hydroxyproline content was higher ($P = 0.001$) in ISA Dual than in Ross 308 chickens. Muscle fiber characteristics were determined using the method by Ashmore and Doerr (1971). The PM of ISA Dual chickens was composed of a higher ($P \leq 0.001$) number of muscle fibers, and since the number of muscle fibers in a 1 mm² area is related to their cross-sectional size, their area was lower ($P \leq 0.001$) than that of Ross 308 chickens, in which the opposite result was obtained. In conclusion, the study found a difference between the genotypes. Dual-purpose cockerels are much more suitable for longer feeding. Although they have a significantly lower proportion of breasts compared to the Ross 308, the breast meat shows better physical and nutritional properties.

Key Words: carcass yield, dual-purpose, genotypes, meat quality, muscle fibers

298 Quality attributes of standard and high breast yielding broilers raised for small and big bird debone markets. Clay J. Maynard*^{GS}¹, Ashunti Jackson², Juan Caldas-Cueva¹, Andy Mauromoustakos³, Michael T. Kidd¹, Samuel J. Rochell¹, Casey M. Owens¹; ¹Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, ²Cobb Vantress, Inc., Siloam Springs, Arkansas, United States, ³University of Arkansas, Fayetteville, Arkansas, United States.

In recent times, meat quality has become a key aspect of broiler production. However, little knowledge is published

in the literature that assesses modern strain, sex, and target weight differences on meat quality. Therefore, the purpose of this study was to assess meat quality variation in strain, sex, and carcass size for four commercially available strains (2 standard yielding (SY) and 2 high yielding (HY)) within the current United States market. In this study, 2,400 sex separate broilers were placed to evaluate performance when grown to two market weights (2.5 and 3.8 kg, representing small bird and big bird program, respectively). Additionally, broilers were fed one of two diets with varying degrees of amino acid inclusion to represent two planes of nutrition. Upon analysis, diet had little impact ($P>0.05$) on parameters and was therefore excluded in further data analysis. A three-way ANOVA was then used to assess various meat quality parameters using main effects of strain, sex, and carcass size. A main effect of strain type was significant for most parameters ($P<0.05$) except for breast width and cook loss ($P>0.05$). For all listed parameters SY strains outperformed those from the HY strains regarding meat quality assessment. Concomitantly, SY strains in the small bird (SB) market performed better than SY strains in the big bird (BB) market as smaller carcasses had lower incidences of white striping, woody breast, spaghetti meat, woody-like tender, and tenderfeathering and improved quality attributes ($P<0.05$). Similar trends were observed ($P<0.05$) in HY strains as SB carcasses produced a better overall product. For each market segment, males had significantly thicker ($P<0.05$) and longer ($P<0.05$) fillets, higher incidences ($P<0.05$) of white striping, and higher ($P<0.05$) cook loss, when compared to females. Differences were also observed in tenderness as SY strain A produced the lowest shear values, whereas SY strain B produced the highest shear values across parameters ($P<0.05$). Differences in carcass size directly impacted quality ($P<0.05$) as SB markets showed improvements in most parameters assessed. Although strain had some impact on quality measures, carcass size and sex had greater impacts in terms of muscle myopathies, water holding capacity, and shear properties. These results suggest that variation in quality at processing in industry may be partly due to processing varying live weights, as well as straight run broilers. Integrators should also strive for a balance between yield and quality.

Key Words: meat quality, myopathies, tenderloin, breast, tenderness

299 Assessment of meat quality attributes of four commercial broiler strains processed at various market weights. Ashleigh J. Mueller¹, Clay J. Maynard*^{GS 1}, Ashunti Jackson³, Juan Caldas-Cueva¹, Aline Giampietro-Ganeco⁴, Xiao Sun², Michael T. Kidd¹, Samuel J. Rochell¹, Casey M. Owens¹; ¹Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, ²Biological Science and Food Engineering, Chuzhou University, Chuzhou, Anhui, China, ³Cobb Vantress, Inc., Siloam Springs, Arkansas, United States, ⁴Animal Science and Food Engineering, University of São Paulo - USP, São Paulo, Brazil.

As the demand for poultry meat continues to grow, the industry is challenged with how to meet these needs of consumers. The current solution is to grow a bigger, higher breast yielding bird that results in increased production at market. The growth rates continually improve in order to increase production efficiently. However, the industry has seen a decline in the quality of the meat as the growth rates and growth periods continue to increase. The objective of this study was to assess changes in quality associated with bird size. In this study, 1,800 broilers from four commercial strains (two high breast yielding (HY) and two standard yielding (SY)) were raised sex separately in order to evaluate meat quality trends over time at six different market weights. Birds were processed at market weights ranging from 2043g to 4313g in 454g increments. All data were subjected to mixed model statistical analysis by sex with fixed effects of strain and carcass size and random effect of pen. High breast yielding strains (HY) had significantly higher breast and tender yields than those of the standard breast yielding (SY) strains ($P<0.05$). There was an increase in breast and tender yields as target weights increased ($P<0.05$) for both HY and SY strains. Some differences were observed depending on strain for fillet dimensions consisting of length, width, and thickness; however as expected, measurements increased as target weight increased ($P<0.05$). Woody breast (WB) had a higher severity in the HY strains for both males and females ($P<0.05$) when compared to SY strains. There were significant differences for WB and WS ($P<0.05$), showing that over time as target weight increased, severity of the myopathy increased. Spaghetti meat generally decreased ($P<0.05$) from 2497g to 4313g broilers while woody-like tender scores decreased ($P<0.05$) throughout the same range. Tender feathering was inconsistent across strains and weights. For shear values, the main differences were observed among target weight with inconsistent difference between the high and standard yielding strains. Total loss (thaw and cook loss) decreased ($P<0.05$) from 2497g to 4313g in males and females. MORS energy values were significantly different for both sexes among target weights and increased as the target weight increased in the cranial region of the fillet ($P<0.05$). While strain had some significant impact on meat quality attributes, processing weight had a greater impact on quality, specifically muscle myopathies, water holding capacity, and shear values.

Key Words: broiler, market weight, meat quality, myopathy, strain

300 Identification of dead on arrival chickens by analysis of body temperature variation. Montana Riggs*^{GS}, Amrit Pal, Rachel Osborne, Alexandra Jackson, Dianna Bourassa; Poultry Science, Auburn University, Auburn, Alabama, United States.

Chickens dead-on-arrival (DOA) are an animal welfare, food safety and economic concern to poultry. Controlled atmosphere stunning (CAS) utilizes a gradual increase in carbon dioxide to render broilers unconscious before

shackling for slaughter. Because broilers are typically stunned within transportation coops, it can be difficult to differentiate recent DOAs and CAS-stunned birds. In some systems, an entire transport truck is stunned prior to shackling. The objective of this study was to evaluate temperature differentials for identification of DOAs in spent broiler breeders and broilers. To assess broiler breeder DOAs, 24 coops each containing 8-9 spent broiler breeders (n=186) were used. In a separate trial, broiler DOAs were assessed using 24 coops each containing 7 broilers (n=168). One bird from each coop was euthanized and returned to the coop to represent the DOA. The remaining birds were then euthanized after a predetermined time to simulate stunning of the entire coop. The carcasses then remained in the coop for an additional predetermined time period to simulate the time following stunning prior to shackling. The time periods used for the simulated DOA were 1) 15 minutes, or 2) 120 minutes. The time periods used to simulate time between coop stunning and shackling were 1) 5 minutes, 2) 30 minutes, 3) 60 minutes, or 4) 120 minutes. At each "post-stun" time, body temperatures were recorded using thermal imaging (broiler breeder and broilers) and an infrared thermometer (broilers only). Temperature comparisons between the DOA and the coopmates were analyzed using one-way ANOVA with means separated by Tukey's HSD. Values were considered significant when the P value was ≤ 0.05 . For broiler breeders, only birds that had been dead for 120 minutes (30.4°C) were distinguishable from coopmates (34.4°C) when temperature was evaluated 5 minutes post "stun" ($p < 0.0001$). However, for broilers, all treatment groups with DOA birds that had been dead for 120 minutes followed by entire coop euthanasia times at 120, 60, 30, and 5 minutes were found to be distinguishable ($P = 0.0186, 0.0475, 0.0339, 0.0066$), with body temperatures of the simulated DOA birds recorded at 27.4°C, 26.7°C, 29.3°C, 28.3°C in comparison to the coopmates at 29.4°C, 28.9°C, 31.1°C, 31.6°C. Neither trial resulted in significant differences of body temperatures with a DOA time of 15 minutes. It would be valuable to determine the shortest distinguishable DOA time. Using temperature differentials between coopmates for identifying DOAs shows potential, particularly for stunning systems in which an entire transport trailer is stunned prior to shackling, therefore leading to an extended time period between stunning and shackling.

Key Words: broiler breeder, broiler, dead on arrival, time, temperature

301 The effect of High Voltage Atmospheric Cold Plasma (HVACP) on chicken egg quality. Nooshin Nikmaram, Abrar Alzaydi, Aarushi Bhardwaj, Alba E. Illera, Linyi Tang*, Vanessa R. Souza, Kevin Keener; Engineering, University of Guelph, Guelph, Ontario, Canada.

High Voltage Atmospheric Cold Plasma (HVACP) is an in-package, rapid and effective non-thermal technique that has gained recent attention due to its low costs and simple

design. The HVACP ionizes the gas inside a sealed package into reactive gas species (RGS) such as ozone (O_3) which have bactericidal properties. Previous studies have shown greater than 5 logs reductions of *Salmonella Enteritidis* (SE) on egg shell after a few minutes of cold plasma treatment. However, it is crucial to investigate the effect of HVACP on egg quality. Therefore, this study was undertaken to evaluate the effect of HVACP on chicken egg quality using treatments previously reported to demonstrate greater than 5 log reductions of SE. The quality analyses performed included: egg shell thickness, Haugh unit (HU), height of albumen, color, pH, egg shell strength, and vitelline membrane strength (VMS). The investigation evaluated egg quality during four weeks of post-treatment storage under refrigerated conditions. Grade A, large chicken eggs, estimated to be seven to ten days old, were purchased from the local grocery store. The eggs were randomized and grouped into three egg sets. Each set of eggs was then sealed in a 6.5 L container in dry air (15% RH) and treated with HVACP at 90 kV under direct and indirect mode of exposure for 5 and 15 minutes with no post-treatment storage. All testing was performed in triplicate. Each egg quality analysis included nine egg measurements per experimental condition. Statistical analysis was performed using ANOVA statistical analysis software with a $P < 0.05$ indicating statistical significance. Results found that treated and untreated eggs remained at 'A' grade or better after two weeks of refrigerated storage with no significance difference in Grade between treatment conditions. Additionally, no significant difference was observed in terms of shell thickness, HU, height of albumen, color, pH, and egg shell strength between treated and control samples. As expected from previous literature the VMS significantly (P -value: 0.027) decreased during the two weeks of storage from 0.03 to 0.02 N in both the control eggs and treated eggs. These results indicate that HVACP treatment up to 15 minutes under direct treatment did not affect egg quality during an additional two weeks of storage.

Key Words: High voltage cold plasma, chicken egg, quality parameters

302 Textural analysis of pet treats developed from broiler chicken Wooden Breast meat and carcass frame combinations. Gerardo A. Abascal-Ponciano*^{GS 1}, Marc R. Presume¹, Moses Chilenje¹, Joshua J. Flees¹, Jorge L. Sandoval¹, Jason T. Sawyer², Barney S. Wilborn², Robert P. Mason³, Eric K. Altom³, Charles W. Starkey¹; ¹Poultry Science, Auburn University, Auburn, Alabama, United States, ²Animal Science, Auburn University, Auburn, Alabama, United States, ³Balchem Animal Nutrition and Health, Balchem Corp., New Hampton, New York, United States.

Co-products of broiler chicken processing, like carcass frames (GF), are considered low-value products. Wooden breast- (WB) affected fillets are often downgraded and sold at a reduced price. These co-products have potential to increase their value through transformation into high-priced

goods. Pet treats are valuable items that can use these products as rich protein source. Addition of sodium alginate and encapsulated calcium lactate (ALGIN) can act as gelling agents for these mixtures. The objective of this study was to assess textural parameters of pet treats developed from 5 different GF:WB mixtures with 2 ALGIN inclusions. Severe (score 3) WB and GF were each ground using a Thompson 3000 industrial grinder and mixed in the following ratios: GF0:WB1, GF1:WB3, GF1:WB1, GF3:WB1, GF1:WB0. Mixtures were combined with 1 of 2 ALGIN concentrations (0.500% of sodium alginate + 0.425% encapsulated calcium lactate (0.5x), and 1.00% of sodium alginate + 0.85% encapsulated calcium lactate (1x) to obtain 10 treatments. Each treatment was extruded into a 52-mm diameter casing, wrapped in plastic wrap, and refrigerated at 1 °C for 16 h and frozen at -11 °C for 3 h prior to being sliced into 25-mm thick slices. Slices were cooked with a MIWE ECONO Convection oven at 93 °C with a fan speed at 2 for 2 h (target internal temperature 75°C). Texture profile analysis (TPA) measures: hardness (maximum force required to deform), cohesiveness (represents structural integrity under compression), adhesiveness (adhesion of sample to the probe), chewiness (energy required to masticate), resilience (ability of sample to regain original form), and springiness (height that the food recovers between compressions). Ten slices per treatment underwent TPA using a two-cycle compression with aTA-25 cylinder probe and a test speed of 5 mm per s. Data were analyzed using SAS (v9.4) PROC GLIMMIX and least square means were separated using PDIFP at $P \leq 0.05$. An ALGIN \times GF:WB ratio interaction was observed for all variables measured, except adhesiveness. Hardness of samples was inversely proportional to increasing GF and ALGIN inclusion ($P < 0.0001$). Resilience and cohesiveness were reduced with increasing concentrations of ALGIN except for the GF1:WB0 ratio ($P = 0.0002$). Springiness was reduced for all ratios when ALGIN inclusion increased to 1x ($P < 0.0001$) but with varying magnitudes. Chewiness of slices increased with 0.5x ALGIN inclusion, decreased as WB inclusion decreased, and was highest for GF0:WB1 mixture with 0.5x ALGIN ($P < 0.0001$). Adhesiveness was similar among all treatments ($P = 0.5775$). In conclusion, the processing co-products were successfully transformed into pet treats, increasing potential profitability.

Key Words: alginate, carcass frame, pet treat, texture, Wooden Breast meat

303 Textural characteristics analysis of pet treats generated from mixtures of ground chicken carcass frames and wooden breast meat. Orlando B. Fiallos*^{UG1}, Marc R. Presume¹, Moses Chilenje¹, Gerardo A. Abascal-Ponciano¹, Joshua J. Flees¹, Jorge L. Sandoval¹, Barney S. Wilborn², Jason T. Sawyer², Robert P. Mason³, Eric K. Altom³, Charles W. Starkey¹; ¹Poultry Science, Auburn University, Auburn, Alabama, United States, ²Animal Science, Auburn University, Auburn, Alabama, United States, ³Balchem Animal Nutrition and Health, Balchem Corp., New Hampton, New York, United States.

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Pet food is a rapidly growing, large commercial food product industry that can utilize high quality, low-cost broiler chicken processing co-products such as carcass frames (GF) and Wooden Breast meat (WB) to generate highly marketable pet treats. Textural characteristics of pet food products are important and can influence purchasing decisions of consumers. The objective of this study was to evaluate textural characteristics (TC) of pet treats generated from mixtures of GF and WB. All products were ground using a Thompson 3000 Industrial Mixer/Grinder. GF was first ground with a (9.52-mm) then with a grinder plate (4.76-mm) and WB was ground with a (4.76-mm) grinder plate. These ground products were mixed to achieve the following 5 ratios: 0% GF:100% WB, 25% GF:75 %WB, 50% GF:50% WB, 75% GF:25% WB, 100% GF:0% WB. Subsamples of the 5 GF:WB mixtures were then mixed with 2 different dosages (50 or 100% of the recommended dosage) of sodium alginate and encapsulated calcium lactate (ALGIN; 0.85% calcium lactate and 1% sodium alginate). Combinations of GF-WB and ALGIN dosages resulted in a total of 10 treatments. Weighing, grinding, and mixing of the products were performed at 4 °C. Each treatment was extruded into a 52-mm diameter casing, wrapped in plastic wrap, and refrigerated at 1 for 16 h and frozen at -11 °C for 3 h prior to being sliced into 3-mm thick slices for TC analysis. Slices were cooked in a 149 °C Koch smokehouse (target internal temperature 74 °C; 6-8 min). Oven temperature was decreased to 66 °C and samples were cooked until water activity reached 0.80 (4 h). Stiffness, hardness, and flexibility of dehydrated pet treats were analyzed using the three-point bending test. Data were analyzed with the GLIMMIX procedure of SAS (V9.4) and least square means were separated using the PDIFP option at $P \leq 0.05$. Interactions of the main effects of GF:WB ratio and ALGIN inclusion were observed for all TC parameters measured. Stiffness was not affected by ALGIN inclusion ($P = 0.2123$); however, stiffness increased as WB proportion increased ($P < 0.0001$). An interaction ($P < 0.0001$) between GF:WB ratio and ALGIN dosage was observed for hardness and as WB inclusion decreased, increasing ALGIN to 1X resulted in a lower increase in hardness. Flexibility increased as GF proportion increased ($P = 0.2394$); however, increasing dosages of ALGIN were not equal across all combinations. In conclusion, the processing of broiler co-products is a feasible way to add value to these ingredients and manufacture quality pet treats. Combination of GF and WB with ALGIN to manufacture pet products can provide economically benefit the poultry industry.

Key Words: textural characteristics, broiler chicken processing, Wooden Breast meat, alginate, pet food

304 Textural profile analysis of raw pet treats generated from mixtures of carcass frames and wooden breast meat. J. Enrique Banegas*^{UG1}, Marc R. Presume¹, Moses Chilenje¹, Gerardo A. Abascal-Ponciano¹, Joshua J. Flees¹, Jorge L. Sandoval¹, Barney S. Wilborn², Jason T. Sawyer², Robert P. Mason³, Eric K. Altom³, Charles W.

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Wooden Breast (WB) meat and carcass frames (GF) are co-products generated during broiler processing. These products are often downgraded due to poor quality, resulting in economic losses for producers. However, they have a great potential in the creation of new products in the pet treat industry. In the last few decades, the pet food industry has experienced massive growth. Texture is an organoleptic characteristic that influences consumers' buying decisions. The objective of this study was to evaluate textural characteristics of pet treats generated from mixtures of GF and WB with inclusion of sodium alginate and encapsulated calcium lactate (ALGIN). Carcass frames and severely affected Wooden Breast meat were ground and mixed to obtain 5 different combinations 0%GF:100%WB, 25%GF:75%WB, 50%GF: 50%WB, 75%GF:25%WB, 100%GF:0%WB. Samples were mixed with 2 ALGIN inclusions at 50 and 100% of the manufacturer's recommended dosage of ALGIN (100% ALGIN = 0.85% encapsulated calcium lactate and 1% of sodium alginate). Each treatment was extruded into a 52-mm diameter casing, wrapped in plastic wrap, and refrigerated at 1 °C for 16 h and frozen at -11 C for 3 h prior to being slicing into 25-mm thick slices. Texture Profile Analysis (TPA) measures: hardness (maximum force required to deform), cohesiveness (represents structural integrity under compression), adhesiveness (adhesion of sample to the probe), chewiness (energy required to masticate), resilience (ability of sample to regain original form), and springiness (height that the food recovers between compressions). Data were analyzed using the GLIMMIX procedure of SAS (v9.4) and least square means were separated using the PDIF option at $P \leq 0.05$. An interaction was observed between GF:WB combinations and ALGIN inclusions for all the variables measured. Hardness increased as WB combinations and ALGIN inclusion increased ($P < 0.0001$). Treats were more adhesive as GF and ALGIN increased ($P = 0.0013$). Resilience of raw samples decreased with increasing GF but increased as ALGIN increased ($P < 0.0001$). Cohesiveness values peaked at 100%GF:0%WB and 0%GF:100% WB but were the lowest at 50%GF:50%WB. Treats were also more cohesive as ALGIN increased ($P < 0.0001$). Springiness of raw treats peaked at 100%GF:0%WB and 0%GF:100% WB but were the lowest at 75%GF:25%WB. Springiness decreased with decreasing ALGIN inclusion ($P = 0.0036$). Chewiness decreased as GF increased, but treats were chewier as ALGIN inclusion increased ($P < 0.0001$). In conclusion, the low value co-products were successfully used to produce pet treats. Generating high quality products ensures a greater economic return in the poultry processing industry.

Key Words: Wooden Breast meat, carcass frame, texture analysis, pet treats, broiler chicken processing co-products.

305 Evaluation of the texture profile analysis of pet treats generated from mixtures of broiler liver and heart. Christopher I. Almendares*^{UG 1}, Marc R. Presume¹, Rigo F. Soler¹, Moses Chilenje¹, Jorge L. Sandoval¹, Luis P. Avila¹, Laura J. Garner¹, Amit Morey¹, Robert P. Mason², Eric K. Altom², Charles W. Starkey¹; ¹Poultry Science, Auburn University, Auburn, Alabama, United States, ²Balchem Animal Nutrition and Health, Balchem Corp., New Hampton, New York, United States.

Broiler chicken liver (CL) and chicken heart (CH) are often undervalued co-products which could lead to new pet food products. Characteristics, such as texture, determine the quality of a meat product. An experiment was designed to evaluate raw meat blends as pet food treats from chicken co-products (CL and CH), as well as additions of sodium alginate and encapsulated calcium lactate (ALGIN) as a structuring agent. The objective of this study was to evaluate texture profile analysis (TPA) of pet treats generated from 3 mixtures of raw CL and CH with 2 ALGIN inclusions. CL and CH were ground individually with a 4.76-mm plate. Products were mixed at 3 ratios of CL and CH: 25%CL:75%CH, 50%CL:50%CH, and 75%CL:25%CH. Subsamples of the 3 CL:CH mixtures were then mixed with 0.5x (0.425% encapsulated calcium lactate and 0.5% sodium alginate) and 1x (0.825% encapsulated calcium lactate and 1% sodium alginate) ALGIN inclusion to produce final batches ($n = 6$), stuffed into 69-mm casings, and refrigerated at 3°C for 48 h prior to slicing. Meat products were cut into 25-mm-thick slices for TPA. Hardness, springiness, cohesiveness, adhesiveness, chewiness, and resilience of samples were measured using 2-cycle compression with a TA-40-cylinder probe. Data were analyzed with the GLIMMIX procedure of SAS and means were separated at $P \leq 0.05$. Interactions were observed for all variables evaluated ($P \leq 0.0001$). Hardness increased as CL proportion decreased ($P \leq 0.0001$), with 1X ALGIN inclusion expressing the highest values for hardness ($P \leq 0.0001$). In relation to the other treatments, adhesiveness was lowest in the 50%CL:50%CH + 0.5x ALGIN samples ($P \leq 0.0001$). Values for resilience were higher for all samples containing 1x concentrations of ALGIN ($P \leq 0.0001$) with inconsistent values measured for mixtures utilizing 0.5x ALGIN. Cohesiveness declined ($P \leq 0.0001$) with increasing concentrations of CL, but only in the 1x ALGIN inclusions. Springiness was highest ($P \leq 0.0001$) when 50%CL:50%CH are mixed with 0.5x ALGIN inclusion and lowest ($P \leq 0.0001$) in the 50%CL:50%CH + 1x ALGIN inclusion. Chewiness increased ($P \leq 0.0001$) at 1x ALGIN inclusion and was highest ($P \leq 0.0001$) in 25%CL:75%CH + 1x ALGIN samples. In conclusion, TPA results from this study demonstrated that co-products from poultry meat processing can be mixed with varying ALGIN inclusions to produce pet food products. Utilizing the right amounts of each co-product could lead to better quality products, better consumer acceptance and could reduce production costs.

Key Words: alginate, broiler chicken processing, textural

characteristics, pet food

306 Textural characteristics of pet treats generated from mixtures of broiler chicken liver and heart. Diego E. Ventura*^{UG1}, Marc R. Presume¹, Rigo F. Soler¹, Moses Chilenje¹, Jorge L. Sandoval¹, Luis P. Avila¹, Laura J. Garner¹, Amit Morey¹, Robert P. Mason², Eric K. Altom², Charles W. Starkey¹; ¹Poultry Science, Auburn University, Auburn, Alabama, United States, ²Balchem Animal Nutrition and Health, Balchem Corp, New Hampton, New York, United States.

Processing chicken generates co-products with reduced commercial value, of these, chicken liver (CL) and chicken heart (CH), both possess great potential for generating highly nutritious products. Pet treats are one alternative way to add value to meat industry co-products. New meat products as pet treats need to be evaluated for different characteristics prior to moving to the development phase. Texture is one characteristic that needs to be evaluated in new products to determine quality. Shear force (SF) is a texture analysis that measures the firmness and toughness. Firmness is the amount of force needed to slice the product and toughness is the accumulated effort needed to slice the product. The objective of this study was to evaluate the SF of pet treats generated from mixtures of CL and CH with additions of different dosages of ALGIN (structure forming agent made of sodium alginate and encapsulated calcium lactate). Initially, raw CH and CL were ground separately to 4.76 mm, and then mixed into 3 different ratios of CL to CH: 25%CL:75%CH, 50%CL:50%CH, 75%CL:25% CH. Mixtures were divided and blended with 1 of 2 different concentrations of ALGIN; 1x (1.00% sodium alginate + 0.85% encapsulated calcium lactate) and 0.5x (0.50% sodium alginate + 0.42% encapsulated calcium lactate) to produce final batches. Each treatment was extruded using a commercial stuffer to produce 20-mm-thick samples, which were wrapped in plastic and stored at 3 °C for 48 h. Each sheet was cut into 25.4 mm width × 63.5 mm length × 20 mm thick strips and dehydrated at 93 °C for 2.5 h. Toughness and Firmness were analyzed with the TA-42 blade, a 3-mm-thick, 7-cm wide and 45° chisel-edged blade. The blade moved at 1.5 mm per s until it passed through 95% of the product. Data were analyzed using the GLIMMIX procedure of SAS (v9.4) and least square means were separated using the PDIF option at $P \leq 0.05$. For both firmness and toughness interactions among CL:CH ratio and ALGIN dosage were observed ($P \leq 0.0001$). Samples containing 50%CL:50%CH + 0.5x ALGIN had the highest firmness values compared with all other combinations including 1x ALGIN inclusions ($P \leq 0.0001$). Combinations of 25%CL:75%CH + 0.5x ALGIN had the highest toughness values, while the 75%CL:25%CH + 0.5x ALGIN had the lowest values ($P \leq 0.0001$). All remaining samples were intermediate regardless of the concentration of ALGIN or CL:CH ratio. In conclusion, the results from this experiment demonstrated that both CL and CH can be effectively combined with ALGIN and used as primary ingredients to produce pet treats, thereby enhancing the

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economic value of these broiler industry co-products.

Key Words: shear force, broiler chicken processing, co-products, pet food, alginate

307 Evaluation of three-point bend textural characteristics of pet treats created from the combination of different chicken processing co-products. Allan J. Calderon*^{GS1}, Marc R. Presume¹, Rigo F. Soler¹, Moses Chilenje¹, Jorge L. Sandoval¹, Luis P. Avila¹, Laura J. Garner¹, Amit Morey¹, Robert P. Mason², Eric K. Altom², Charles W. Starkey¹; ¹Poultry Science, Auburn University, Auburn, Alabama, United States, ²Balchem Animal Nutrition and Health, Balchem Corp., New Hampton, New York, United States.

Co-products obtained from broiler processing may end up being used in lower value products or simply rendered as their value is often underestimated. However, there is a great opportunity for poultry meat producers to utilize these co-products to create new products, and thus, maximize their economic return. Texture characteristics of pet food treats can often completely define buyer preference for a specific product. Therefore, the objective of this randomized complete block design experiment with a 3×2 factorial treatment arrangement was to evaluate textural characteristics of jerky-type pet treats generated from 3 mixtures of chicken liver (CL) and chicken heart (CH) and 2 concentrations of encapsulated calcium lactate (ECL) and sodium alginate (SA). Different proportions of raw CL and CH were ground separately through a 4.76-mm grinder plate, then combined to achieve a total of 3 mixtures. The mixtures generated were: 25%CL:75%CH, 50%CL:50%CH, and 75%CL:25%CH. ALGIN is a structure forming agent composed of ECL and SA. The ALGIN concentrations added to the CL:CH mixtures were: 1x(0.85% of ECL and 1% of SA) and 0.5x (0.425% of ECL and 0.5% of SA) of the manufacturer's recommended dosage. The 6 different combinations of raw product were extruded and shaped to create sheets of product which were then covered with plastic and stored at 3 °C for 48 h. After 48 h of refrigeration, product layers were sliced into 25.4 mm width × 63.5 mm length × 20-mm-thick, jerky-type pet treats and allocated in metal trays. Pet treats were dehydrated at 93°C for 2.5 h and then left to cool to room temperature. Subsequent analyses of stiffness, hardness, and flexibility were performed using 10 samples per treatment with a TA-92 three-point bend apparatus. Data were analyzed as using the GLIMMIX procedure of SAS (v9.4) and least squares means were separated using the PDIF option at $P \leq 0.05$. Stiffness was higher on 50%CL:50%CH + 1x ALGIN compared with all other treatments ($P \leq 0.0001$). However, the 75%CL:25%CH combination + 0.5x ALGIN had greater stiffness compared with other treatments at the same ALGIN dosage ($P \leq 0.0001$). Also, pet treats of 50%CL:50%CH + 1x ALGIN concentration had greater hardness ($P = 0.0043$). The lowest flexibility was observed in 75%CL:25%CH + 0.5x ALGIN samples ($P = 0.0295$). In conclusion, these results

demonstrate that co-products from poultry meat processing can be successfully utilized to produce pet food products. Maximizing the utilization of these co-products has the potential to generate another important source of income to poultry meat producers.

Key Words: chicken liver, chicken heart, alginate, pet food

308 Effect of inclusions of a forming agent on texture analysis of pet treats developed from cooked chicken paws.

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It is important to add value to broiler co-products, such as chicken paws (CP). ALGIN is a combination of two functional ingredients: sodium alginate and encapsulated calcium lactate and can be used to provide a more stable structure to the boiled ground CP products and achieve a much higher retail value through incorporation into pet treats. The objective of this study was to assess the effect of different inclusions of forming agent (ALGIN) on texture profile analysis (TPA) and shear force (SF) of CP-derived pet treats. Previously frozen CP were pressure cooked with 13% added water using a GoWISE electric pressure cooker at 13 psi for 90 min. Total cooking moisture loss was 20.16%. Cooked CP were ground using a 9.52-mm and then a 4.76-mm grinder plate. ALGIN was added and mixed with ground CP at 3 concentrations: 0.5x (0.50% sodium alginate + 0.425% encapsulated calcium lactate), 1x (1.00% sodium alginate + 0.85% encapsulated calcium lactate), and 2x (2.00% sodium alginate + 1.70% encapsulated calcium lactate). After mixing, each treatment was extruded into 21-mm diameter casings, stored at -15°C for 16 h, and sliced into 20-mm thick slices for texture analysis. Shear force test was performed using a TA-42 45° chisel blade, to measure toughness (total force over distance) and firmness (peak force). The TPA measures: hardness (maximum force required to deform), cohesiveness (represents structural integrity under compression), adhesiveness (adhesion of sample to the probe), chewiness (energy required to masticate), resilience (ability of sample to regain original form), and springiness (height that the food recovers between compressions). Measurements were conducted with a two-cycle compression with a TA-25 cylinder probe and a test speed of 5 mm per s. Data were analyzed using the GLIMMIX procedure of SAS (v9.4) and least square means were separated using the PDIF option at $P \leq 0.05$. When performing the SF test, firmness was similar for 0.5x and 1x ALGIN inclusions and 2x ALGIN inclusion samples were less firm ($P = 0.0232$). Shear force toughness was higher with 0.5x and 1x ALGIN inclusions compared with

2x ALGIN inclusion ($P = 0.0004$). From TPA, hardness, resilience, cohesiveness, springiness, and chewiness values decreased as ALGIN inclusion increased in cooked CP products ($P < 0.0001$). Samples from the 0.5x and 1x ALGIN inclusion treatments had lower adhesiveness values than those from the 2x ALGIN inclusion treatment ($P = 0.0017$). In conclusion, co-products like cooked CP can be successfully incorporated into higher value products for the retail pet food industry. These results indicate that ALGIN inclusion allowed cooked proteins to be formed and sliced.

Key Words: chicken paws, alginate, broiler processing co-products, texture analysis

309 Evaluation of color change over time in pet treats produced from chicken paws using sodium alginate and encapsulated calcium lactate as a stabilizer.

Jorge R. Romero*^{UG 1}, Marc R. Presume¹, Said J. Herrera¹, Joshua S. Renew¹, Haisten R. Smith¹, Jorge L. Sandoval¹, Theodore B. Turall¹, J. Enrique Banegas¹, J. Wesley Rogers¹, Orlando B. Fiallos¹, Gerardo A. Abascal-Ponciano¹, Cristopher I. Almendares¹, Diego E. Ventura¹, Amit Morey¹, Laura J. Garner¹, Robert P. Mason², Eric K. Altom², Charles W. Starkey¹; ¹Poultry Science, Auburn University, Auburn, Alabama, United States, ²Balchem Animal Nutrition and Health, Balchem Corp., New Hampton, New York, United States.

The global poultry industry is a fast-growing market that was estimated to have reached \$99 billion USD in 2020. It produces vast amounts of undervalued products such as chicken paws (CP) that can be processed to make other products like pet treats with greater retail value. Therefore, the objective of this study is to measure how different inclusions of ALGIN (structure forming component formed by sodium alginate and encapsulated calcium lactate) affect color in CP pet treats over time (0, 3, 5 and 7 d). A GoWISE electric pressure cooker at high pressure (13 PSI) was used to cook CP for 90 min. Cooked CP were ground twice, with a 9.52 and then 4.76-mm grinder plate. Ground, cooked CP was weighed to determine total yield, and divided into three 3,000-g batches. The 3 treatments were as follows: 50% (0.5x), 100% (1x), and 200% (2x) of the recommended ALGIN concentration (1% sodium alginate + 0.85% calcium lactate). After ALGIN inclusion and mixing, the mixtures were all extruded into a 21-mm diameter casing, covered in plastic wrap, and stored for 16 h at -15 °C prior to being sliced into 10-mm thick slices. Color variations were measured in terms of CIE L*, a*, b* values using the Minolta colorimeter, model CR-300/DP301 (Minolta Corporation, Ramsey, NJ, USA) calibrated with a white calibration plate, a D65 illuminant, 0° viewing angle, and an 8-mm measurement area. Color was measured on 30 sub-samples of each treatment. Data were analyzed with the GLIMMIX procedure of SAS (v9.4) and least square means were separated at $P \leq 0.05$ using the PDIF option. From d 0 to 7, no differences were observed for lightness (L*; $P = 0.3518$). Redness (a*) values decreased as ALGIN inclusion increased ($P = 0.0023$). Redness increased from d 0 to 3 and

stabilized with no further difference observed through d 7 ($P < 0.0001$). Yellowness (b^*) values underwent the most notable change of all color parameters as values decreased from d 0 to 5 and increased again on d 7 ($P < 0.0001$). Increasing ALGIN inclusion increased b^* values ($P < 0.0001$). In conclusion, low value co-products from broiler processing can be utilized post-cooking to manufacture pet treats. However, changes in color over time should be controlled to prevent negative consumer perception and preferences.

Key Words: sodium alginate, calcium lactate, color characteristics, chicken paws, broiler chicken processing

310 Analysis of color variation over time of cooked pet treats made from co-products of broiler chicken processing. Said J. Herrera*^{UG1}, Marc R. Presume¹, Moses Chilenje¹, Gerardo A. Abascal-Ponciano¹, Joshua J. Flees¹, Jorge L. Sandoval¹, Jason T. Sawyer², Barney S. Wilborn², Robert P. Mason³, Eric K. Altom³, Charles W. Starkey¹; ¹Poultry Science, Auburn University, Auburn, Alabama, United States, ²Animal Science, Auburn University, Auburn, Alabama, United States, ³Balchem Animal Nutrition and Health, Balchem Corp, New Hampton, New York, United States.

Broiler processing generates co-products such as Wooden Breast (WB) meat and carcass frames (GF). Pet food manufacturers can leverage these co-products to deliver protein in pet treats. Color of pet treats may influence consumer acceptance. The objective of this study was to evaluate color variations of pet treats generated from WB and GF blends with sodium alginate and encapsulated calcium lactate (ALGIN) inclusions as a protein binding structure-forming component. Co-products were ground using a 4.76-mm grinder plate and mixed to the following ratios: 100% GF: 0% WB, 75% GF: 25% WB, 50% GF: 50% WB, 25% GF: 75% WB, and 0% GF: 100% WB. Subsamples were combined with one of two ALGIN concentrations, $\frac{1}{2}$ x ALGIN (0.5% of sodium alginate + 0.425% encapsulated calcium lactate) or 1x ALGIN (1% of sodium alginate + 0.85% encapsulated calcium lactate) to produce 10 treatments, extruded into 52-mm diameter casings, refrigerated for 16 h at 1 °C, and frozen at -11 °C for 3 h prior to being sliced into 3-mm thick samples. Slices were cooked in a 149 °C Koch smokehouse (target internal temperature 74 °C; 6-8 min). Oven temperature was decreased to 66 °C until water activity reached 0.80 (4 h). Color variations were measured in CIE L^* , a^* , b^* values using the Hunter Lab Miniscan XE Plus colorimeter. L^* means lightness of samples measured from 0 (black) to 100 (white). Values for a^* and b^* ranged from -120 to +120, a^* measures redness from green to red and, b^* measures yellowness from blue to yellow. Three measurements were taken on the surface of each sample on d 0, 3, 5, and 7 post-slicing. Data were analyzed using the GLIMMIX procedure of SAS (v9.4) and least square means were separated using the PDIF option at $P \leq 0.05$. An interaction among GF:WB ratio and ALGIN inclusion was observed for a^* and b^*

values but not for L^* . Samples with lower GF concentration were lighter ($P < 0.0001$). Lightness was not affected by ALGIN inclusion ($P = 0.799$). Lightness also increased from d 0 to 3, then from d 5 to 7 ($P < 0.0001$). Redness decreased as GF concentration increased ($P < 0.0001$) but increased as ALGIN inclusions decreased ($P < 0.0001$). Redness values increased from d 0 to 3, then decreased on d 5 and increased on d 7 ($P < 0.0001$). Yellowness decreased as GF concentration increased ($P < 0.0001$). Treats were more yellow as ALGIN inclusion decreased ($P < 0.0001$). Yellowness increased from d 0 to 3, then decreased on d 5 ($P < 0.0001$). In conclusion, color is an important variable for consumer pet treat buying preferences. While color changes were observed, the inclusion of structure forming technologies such as ALGIN may benefit broiler processors in adding value to co-products.

Key Words: Wooden Breast meat, sodium alginate, carcass frame, water activity, pet treats

311 Evaluation of color variation of raw pet food treats made from broiler chicken processing co-products. Joshua S. Renew*^{UG1}, Marc R. Presume¹, Moses Chilenje¹, Gerardo A. Abascal-Ponciano¹, Joshua J. Flees¹, Jorge L. Sandoval¹, Jason T. Sawyer², Barney S. Wilborn², Robert P. Mason³, Eric K. Altom³, Charles W. Starkey¹; ¹Poultry Science, Auburn University, Auburn, Alabama, United States, ²Animal Science, Auburn University, Auburn, Alabama, United States, ³Balchem Animal Nutrition and Health, Balchem Corp, New Hampton, New York, United States.

Wooden Breast (WB) meat and carcass frames (GF) are co-products that originate from processing of broiler chickens. Myopathies such as WB cause defects in the texture of the pectoralis major muscle, resulting in an inferior product unsuitable for sale for human consumption or refusal by consumers. Pet treats are a great opportunity to use WB and GF to maximize economic profits. When consumers are buying pet treats, one attribute they may use to differentiate between products is color. The objective of this experiment was to assess the effect of increasing additions of sodium alginate and encapsulated calcium lactate (ALGIN) on jerky-style pet treat color variation (CV). ALGIN is a structuring agent used in food products. An experiment was designed with different proportions of WB and GF and color was measured over 7 d post-production. Severe (score 3) WB fillets and GF mixed as follows: 0% GF- 100% WB, 25% GF-75% WB, 50% GF-50% WB, 75% GF-25% WB, and 100% GF-0% WB. Subsamples were made using 2 different concentrations of ALGIN. Concentrations included 0.5% and 1% sodium alginate with 0.425% and 0.85% encapsulated calcium lactate, respectively. Each treatment was stuffed into a 52-mm diameter casing, wrapped in plastic wrap, and refrigerated at 1 °C for 16 h and frozen at -11 °C for 3 h prior to being sliced into 3-mm thick slices. Raw samples were analyzed for Lightness (L^*), Redness (a^*), and Yellowness (b^*). L^* of samples was measured on a scale of 0 to 100. Zero represents the color black while 100

represents white. Redness and yellowness were represented with values that range from -120 to +120. a^* varies from green to red, and b^* varies from blue to yellow. All measurements were taken using a Minolta colorimeter on raw treats d 0, 3, 5, and 7 post-production. The CV were measured on the surface of the sample that remained exposed. Data were analyzed using the GLIMMIX procedure of SAS (v9.4) and the PDIF option was used to separate least square means at $P \leq 0.05$. An interaction was observed among GF:WB ratio and ALGIN inclusion for color ($P < 0.0001$). Raw treat lightness decreased from d 0 to 5 and increased on d 7 ($P < 0.0001$). Values for L^* increased as WB concentrations increased and ALGIN percentage increased ($P < 0.0001$). Redness decreased from d 0 to 7, increased as ALGIN decreased ($P < 0.0001$), and declined when WB concentration was reduced except for the 0%GF-100%WB ratio. Yellowness decreased from d 0 to 7 and with decreasing WB concentrations ($P < 0.0001$) and increased as ALGIN concentration increased ($P < 0.0194$). These results suggest that co-products considered inferior products can now have a larger economic impact as pet treats in the pet food industry.

Key Words: color variation, broiler chicken processing, Wooden Breast meat, alginate, pet treat

312 Novel structure forming technology for the manufacturing of pet treats utilizing broiler chicken paws. Haisten R. Smith*^{UG1}, Marc R. Presume¹, Said J. Herrera¹, Joshua S. Renew¹, Jorge L. Sandoval¹, Theodore B. Turall¹, J. Enrique Banegas¹, J. Wesley Rogers¹, Orlando B. Fiallos¹, Gerardo A. Abascal-Ponciano¹, Cristopher I. Almendares¹, Diego E. Ventura¹, Laura J. Garner¹, Amit Morey¹, Robert P. Mason², Eric K. Altom², Charles W. Starkey¹; ¹Poultry Science, Auburn University, Auburn, Alabama, United States, ²Balchem Animal Nutrition and Health, Balchem Corp., New Hampton, New York, United States.

Chicken paws are one part of the bird that are not typically used in human foods. Utilization of chicken co-products in pet food could add significant value to chicken paws and increase profits in the poultry industry. Paws that are not fit for human consumption are often downgraded and sold for meal production at a lower value. It is of economic importance to utilize as much as possible of the chicken to improve the economic return. Additionally, while rendering is an extremely sustainable method of converting co-products for repurposing, higher value markets may offset additional costs of producing broiler chickens. Co-products in broiler processing such as chicken paws may exhibit difficulties such as textures that can be difficult to work with. In addition, structure forming technologies have been well documented in raw meat products but information regarding their efficacy in cooked meats is limited. The objectives of this experiment were to evaluate the impact of sodium alginate and encapsulated calcium lactate (ALGIN) inclusion on the chemical characteristics of treats produced from cooked, ground chicken paws. Chicken paws (bone-

in) were pressure cooked in an electric pressure cooker for 90 min at 13 psi and ground using a 9.52 and then a 4.76-mm grinder plate. Paws were weighed prior to and after pressure cooking, to determine the loss of moisture which was 20.16%. Samples were then mixed with 3 different concentrations of ALGIN (0.5% of sodium alginate + 0.425% calcium lactate; 1% of sodium alginate + 0.85% calcium lactate; 2% of sodium lactate + 1.74% of calcium lactate) to produce 3 treatments. Each treatment batch was extruded into 21-mm diameter casings, covered in plastic wrap, and stored for 16 h at -15 °C prior to being sliced into 10-mm thick slices. Thirty sub-samples of each treatment were used for analysis for each of the following, expressible moisture, pH, and water activity. Data were analyzed using the GLIMMIX procedure of SAS (v9.4) and means were separated using the PDIF option at $P \leq 0.05$. Expressible moisture of pressure-cooked treats was not affected by increasing concentrations of ALGIN ($P = 0.6784$). Differences were also not observed for pH regardless of ALGIN concentration ($P = 0.3041$). Additionally, ALGIN concentration had no effect on water activity ($P = 0.3041$). These data suggest that lower value co-products can be utilized to produce acceptable pet treat products. Use of lower valued co-products may add significant value to the broiler processing industry. Finally, structure forming technologies previously used with raw products may be utilized with cooked proteins.

Key Words: chicken paws, alginate, pet food, broiler chicken processing, co-products

313 Chemical analysis of pet treats generated using varying combinations of broiler processing co-products. J. Wesley Rogers*^{UG1}, Marc R. Presume¹, Moses Chilenje¹, Gerardo A. Abascal-Ponciano¹, Joshua J. Flees¹, Jorge L. Sandoval¹, Barney S. Wilborn², Jason T. Sawyer², Robert P. Mason³, Eric K. Altom³, Charles W. Starkey¹; ¹Poultry Science, Auburn University, Auburn, Alabama, United States, ²Animal Science, Auburn University, Auburn, Alabama, United States, ³Balchem Animal Nutrition and Health, Balchem Corp., New Hampton, New York, United States.

Wooden breast (WB) has a negative economic impact on the poultry industry due to its undesirable texture and inability of the meat to be sold as a high value product. This and other lower value poultry co-products, such as carcass frames (GF), are in abundant supply and have the potential to be repurposed into a higher value and more marketable product such as pet treats. The pet treat market is increasing due to the fundamental role pets play in the family nucleus. The objective of this study was to evaluate chemical characteristics when using different combinations of GF, WB, and ALGIN (sodium alginate + encapsulated calcium lactate) to produce jerky style pet treats. WB and GF were ground separately using a Thompson 3000 industrial mixer/grinder before being weighed and mixed with ALGIN. Five different combinations of GF and WB were used as follows: 100%GF:0%WB, 75%GF:25%WB,

50%GF:50%WB, 25%GF:75%WB, and 100%GF:0%WB. Subsamples were made using two combinations of ALGIN to produce 10 total treatments. Inclusion rates of ALGIN were 100% and 50% of the manufacturer's recommended rate (100% ALGIN= 0.85% calcium lactate and 1% sodium alginate 50% ALGIN= 0.425% calcium lactate and 0.5% sodium alginate). Each treatment was stuffed into a 52-mm diameter casing, wrapped in plastic wrap, and refrigerated at 1 °C for 16 h and frozen at -11 C for 3 h prior to being slicing into 3-mm thick slices. Slices were cooked in a 149 °C Koch smokehouse (target internal temperature 74 °C; 6-8 min). Oven temperature was decreased to 66 °C until water activity reached 0.80 (4 h). Samples were analyzed for water activity using a Pawkit Water Activity meter. A Hanna pH meter was used to measure pH of all samples. Expressible moisture was analyzed on raw products using the filter press method. Data was analyzed using the GLIMMIX procedure of SAS (v9.4) and least square means

were separated using the PDIFF option at $P \leq 0.05$. An interaction was observed between GF:WB and ALGIN for all variables. As GF increased pH increased ($P < 0.0001$). ALGIN inclusion had no effect on the pH ($P = 0.6358$). Cooking loss decreased as GF and ALGIN inclusion increased ($P < 0.0001$). Water activity of dehydrated treats increased as GF inclusion increased ($P < 0.0001$). As ALGIN inclusion increased, water activity of dehydrated treats decreased ($P < 0.0001$). 100%GF:0%WB and 0%GF:100% WB had the lowest expressible moisture values, while 75%GF:25%WB had the highest ($P < 0.0001$). As ALGIN inclusion increased, expressible moisture decreased. In conclusion, GF and WB poultry co-products were successfully processed into pet treats that could be sold as a higher retail value product compared with rendering.

Key Words: wooden breast meat, sodium alginate, carcass frame, water activity, pet treats

POSTER PRESENTATIONS

Animal Well-Being and Behavior

314P Laser environmental enrichment increased broiler activity and upregulated myogenic gene expression in the *pectoralis major* muscle. Meaghan M. Meyer*, Anna K. Johnson, Elizabeth A. Bobeck; *Animal Science, Iowa State University, Ames, Iowa, United States.*

Modern broilers are considerably more feed efficient, have improved muscle yields, and reach market weight 4-5 times more quickly than their 1950s predecessors. However, disproportionate upper body growth ultimately outpaces the skeletal system and metabolic capacity of the bird. This leads to a highly inactive animal as well as reduced breast meat quality. While environmental enrichment is widely recognized by broiler producers as a valuable tool to improve animal welfare, options that induce exercise without hindering growth are lacking. Methods to prevent muscle disorders that negatively impact animal welfare as well as poultry meat quality have yet to be established. This work utilized a laser enrichment device previously shown to motivate movement in broilers. The objectives were to increase active behavior and elucidate potential shifts in myogenic gene expression of the *pectoralis major* muscle indicating healthy muscle growth. 1200 Ross 708 broilers were housed in pens of 30 (n=20 pens/treatment), with half of the pens receiving laser enrichment four times daily (LASER; 4-min periods) and the other half serving as the control (CON) for 49d. Seventy focal birds were randomly assigned to 14 video-recorded pens (5 focal birds/video-recorded pen) at the trial's start for behavioral analysis during laser periods d0-8 and wk 1-6. On d42 and 49, the cranial region of the right breast fillet was collected from 15 broilers/treatment/timepoint and RNA was isolated using the RNeasy Fibrous Tissue Kit (Qiagen, Maryland, U.S.). One-Step SYBR Green quantitative PCR (Qiagen) was used to test six myogenic and one housekeeping gene, 28s. Primers included myoblast determination protein 1 (MyoD), myogenin (MyoG), muscle regulatory factor 4 (MRF4), insulin-like growth factor 1 (IGF1), insulin-like growth factor 2 (IGF2), and myostatin (MSTN). Behavior data were analyzed using Proc Glimmix and qPCR data were analyzed using Proc Mixed in SAS Version 9.4 (Cary, N.C.) with the fixed effect of treatment. Over wk 1-6, time spent active was increased in LASER birds in wk 3 (105% increase), wk 4 (214% increase), and wk 5 (193% increase) compared to CON birds at the same timepoints (P<0.05). Percent of time spent at the feeder was increased in LASER birds compared to CON birds in week 4 by 761% (P<0.05). There was significantly upregulated expression of MyoG (P=0.009), MRF4 (P<0.001), IGF1 (P=0.001), and MSTN (P=0.006) in LASER breast tissue compared to CON. These results indicate that laser enrichment not only stimulated voluntary locomotion through wk 5 but caused a shift in myogenic gene expression of the highly valued breast muscle above the control birds that may indicate increased tissue growth and functioning.

Key Words: broiler, behavior, environmental enrichment, breast muscle, meat quality

315P No presentation materials submitted.

316P Laser environmental enrichment increased body

weight and *pectoralis major* muscle width while improving woody breast score in broilers. Meaghan M. Meyer*^{GS}, Anna K. Johnson, Elizabeth A. Bobeck; *Animal Science, Iowa State University, Ames, Iowa, United States.*

The fast growth rate and high breast muscle yield that make modern broilers profitable and sustainable has had unintended side effects on animal welfare and meat quality, namely in terms of leg disorders and woody breast, the predominant breast muscle myopathy. These issues are prevalent in heavier birds nearing market-age but develop earlier in production. Environmental enrichment designed to maintain activity through grow-out is lacking in commercial barns, and as the etiology of woody breast remains unknown, interventions to prevent or minimize muscle damage have yet to be established. Hence, the current study utilized a unique laser device previously shown to stimulate broiler movement. The study objectives were to improve growth performance and breast muscle quality through increased activity and feed intake. Half of 1200 Ross 708 broilers received laser enrichment four times daily (LASER; 4-min periods) and the other half served as control (CON). Performance outcomes were calculated by pen and averaged per bird by each performance period for a 7-wk grow-out. A subset of 200 broilers was measured for breast width on d22, 36, 42, and 49. Woody breast (WB) tactile score and breast fillet weights were collected at d42 and d49 by an individual blinded to treatment. Data were analyzed using Proc Mixed in SAS Version 9.4 (Cary, N.C.) with the fixed effect of treatment. LASER birds had increased body weight at all time-points compared to CON (P<0.001), including d42 (0.14 kg increase) and d49 (0.15 kg increase). Feed conversion ratio was improved in LASER birds by 3 points in the starter period (d0-14; P=0.003). Breast width, measured weekly on live birds beginning on d22, was significantly increased in LASER birds at all time-points (P<0.001), with an increase of 0.48 cm at d42 and 0.47 cm at d49; however, weight of the right breast fillet was not affected by enrichment at d42 nor 49. At d42, 72% of LASER and 60% of CON WB scores were 0 (normal/unaffected), while 8% percent of LASER breasts and 13% of CON breasts were scored 1 (mild WB); remaining scores were intermediate. At d49, 39% of LASER breasts and 24% of CON breasts received a score of 0, 20% of LASER and 26% of CON breasts received a score of 1, and 4% of LASER breasts and 19% of CON breasts were scored 2 (moderate WB); remaining scores were intermediate. The laser enrichment device was successful in consistently improving broiler performance and increasing breast width. Although WB tactile scores were not increased with increased body weight, 12% more LASER birds received a score of 0 on d42 and 15% more scored 0 on d49 vs CON. This is a positive outcome indicating that the laser device is capable of improving performance without causing WB.

Key Words: broiler, performance, environmental enrichment, woody breast, meat quality

317P Study on the distribution feature of angel wing and its relationship with wing bone development, hematological and serum biochemical parameters in

geese. Xinghao Zhu*^{GS}, B. H. Shao, Lin.Ge Gao, H.Y Zhang, W Chen, Y.Q. Huang; *Henan Agriculture University, Henan Province, China.*

Angel wing (AW) is a universal phenomenon of outward twisting along the wrist joint on the unilateral or bilateral wing in birds. AW not only affects the appearance of geese, but also results in flightlessness in the wild birds and compromises the birds' welfare. The aim of this study, therefore, was to reveal the distribution of AW in two separate populations, and studied the association for different wing types with organ index, wing bone development and blood biochemical parameters in geese. As far as 70-day-old Hybrid-Wanxi (HW, N = 700) geese and 150-day-old White Zhedong (ZD, N = 1500) are concerned, the results from student's t-test or chi-square analysis showed that the total incidence of AW was 6.67% in ZD geese and 8.86% in HW geese, respectively. The incidence of AW in bilateral wings (n = 66) was significantly greater than that in unilateral wings (n = 34), and the incidence of AW in left wings (n = 26) was remarkably higher than that in right wings (n = 8) of ZD geese (both $P < 0.01$), but the incidence of AW was similar between unilateral and bilateral in HW geese. The sex has not apparently affected the incidence of AW in both ZD and HW geese. Subsequently, 10 male birds were randomly selected from HW geese (70 days of age) with different wing types to assess the potential relationship between wing types with organ index, bone characteristic or blood biochemical parameters. Data were evaluated using a two-tailed unpaired t-test or the Mann-Whitney U test for normally or non-normally distributed datasets, respectively. The organ index was similar between normal wing (NW) and AW geese. The lengths for the humerus (177.95 mm vs. 173.51 mm), metacarpal (97.12 mm vs. 93.35 mm) and phalanx (41.03 mm vs. 39.11 mm), the phalanx weights (0.46 g/kg vs. 0.41 g/kg), as well as the angle between the humerus and the radial ulna (HRU) in NWs were more pronounced than those in AWs ($P < 0.05$). Furthermore, the platelet size indicators [mean platelet volume (8.80 fL vs. 7.81 fL), platelet ratio (17.46% vs. 10.79%) and platelet distribution width (8.42 fL vs. 6.89 fL)] in NW geese were more pronounced than those in AW geese. Collectively, these findings indicated that AW significantly affected the wing bone length, the phalanx weight and HRU, the occurrence of AW phenotype may be related with dysfunctional platelet activation in geese.

Key Words: angel wing, bone characteristic, blood biochemistry, goose

318P Aviary rearing enhances bone microstructure in Lohmann LSL-Lite and bone mass in Lohmann Brown Lite egg-laying hen pullets compared to conventional cage-rearing. Isabela Vitienes*^{GS 1, 3, 4}, Erin Ross², Alice Bouchard^{3, 4}, Gabrielle Graceffa^{3, 4}, Beatrice Steyn^{1, 3, 4}, Bettina Willie^{1, 3, 4}, Tina Widowski²; ¹*Biological and Biomedical Engineering, McGill University, Montreal, Quebec, Canada,* ²*Animal Biosciences, University of Guelph, Guelph, Ontario, Canada,* ³*Research Centre, Shriners Hospitals for Children Canada, Montreal, Quebec, Canada,* ⁴*Pediatric Surgery, McGill University, Montreal, Quebec, Canada.*

Aviary systems can benefit welfare by providing complex space for activity, given hens are introduced to them early

on to develop needed physiological/behavioural adaptations. A previous study showed that Lohmann Select Leghorn (LSL)-Lite pullets reared in a high-complexity aviary had greater bone density and strength than conventionally reared pullets (Casey-Trott et al., *Poult Sci* 2017). Commercial rearing aviaries differ in their levels of spatial complexity, potentially resulting in varied exposure to mechanical stimuli. We therefore aimed to assess whether pullets reared in aviaries with varying levels of complexity exhibit altered bone mass and microstructure. 5,952 Lohmann Brown-Lite (LB) and LSL-Lite (LW) pullets were reared in conventional rearing cages (CC, n=240/strain) or three different aviary rearing systems with varying levels of complexity (Low (L), n=690/strain; Mod. (M), n=846/strain; High (H), n=1200/strain) until 16 woa, across two consecutive flocks. We assessed bone mass and microstructure in the tibiotarsus diaphysis from a sample of birds (N=141; n=7-13/strain/housing/flock) with μ CT-imaging (15 μ m voxel size). Cortical tissue mineral density (Ct.vTMD) and microstructural parameters (cortical area Ct.Ar, area fraction Ct.Ar/Tt.Ar, thickness Ct.Th, porosity Ct.Po, periosteal and endocortical perimeters Ps.Pm & Ec.Pm) were assessed. Data were analyzed by ANOVA and post-hoc Tukey testing, with significance at $p \leq 0.05$. Housing affected Ct.vTMD, Ct.Ar/T.Ar, and Ct.Th, while flock affected Ct.Ar/T.Ar, Ct.Th, and Ct.Po. The Ct.vTMD, Ps.Pm, Ec.Pm, Ct.Ar/T.Ar, Ct.Th, Imax, and Imin were affected by genetic strain. LB pullets had lower Ct.Th. (-11.1%, -11.8%, -13.4%) and Ct.Ar/T.Ar (-18.3%, -19.3%, -20.6%) than LW pullets reared in L, M and H complexity housing, respectively ($p < 0.0001$). Interestingly, we observed a significant interaction between strain and housing. LW reared in CC had lower Ct.Ar/T.Ar compared to LW reared in L (-11.8%, $p = 0.0002$), M (-11.2%, $p = 0.0012$), and H (-13.6%, $p < 0.0001$) as well as lower Ct.Th when housed in CC compared to L (-5.3%, $p < 0.0001$), M (-5.3%, $p < 0.0001$), and H (-6.5%, $p < 0.0001$). In contrast, LB housed in CC only had lower Ct.vTMD (-5%, $p = 0.0178$) compared to LB housed in L. No differences were observed in either strain between the three aviary systems. Our results confirm reports that rearing in CC is detrimental to bone quality in LSL-Lite pullets. Interestingly, bone microstructure of LB pullets is not affected, indicating that deleterious effects of conventional cage rearing on tibiotarsus microstructure is dependent on strain. We also show that benefits of aviary-rearing in LW and LB pullets is independent of aviary housing complexity examined.

Key Words: Osteoporosis, Pullet, housing, strain, bone

319P The efficacy of increasing dietary L-tryptophan inclusion on broiler breeder pullet growth and fear response. Gabrielle M. House*^{GS 1}, Gregory S. Archer¹, Jason T. Lee²; ¹*Texas A&M University, College Station, Texas, United States,* ²*CJ America - Bio, Downers Grove, Illinois, United States.*

Dietary L-tryptophan, a precursor to serotonin, may reduce undesirable behavioral reactivity associated with feed restriction in broiler breeder pullets; however, an effective inclusion level for L-tryptophan in pullet diets has not been established. Therefore, a study was conducted to evaluate the effects of five dietary tryptophan inclusion levels on broiler breeder pullet growth and fear response. Four-week-old Ross 708 pullets (n = 180) were randomly assigned diets

with one of five supplemental L-tryptophan (Trp) inclusion rates: 0lb/Ton Trp (T0; Control), 1lb/Ton Trp (T1), 2lb/Ton Trp (T2), 4lb/Ton Trp (T3), 8lb/Ton Trp (T4) resulting in dig Trp concentration of 0.10%, 0.15%, 0.20%, 0.30% and 0.49% respectively. All feed was allocated equally across treatments and pullets were feed restricted using a 4:3 feeding schedule for the duration of the 6-week study. All pullets were individually weighed on d51 (23 d post treatment feeding) and d72 (44 d post treatment feeding) to obtain growth measures including body weight (BW), coefficient of variation (CV), and pen uniformity (U). Fear responses of all pullets were assessed via tonic immobility (TI) and inversion (INV) on d53 (25 d post treatment feeding) and d73 (45 d post treatment feeding). All data was analyzed by treatment and by dietary Trp inclusion using One-Way ANOVA where treatment means were considered significant when $P < 0.05$. Pullets fed diets including supplemental tryptophan (T1, T2, T3, and T4) weighed more on d51 and d72 (0.655 ± 0.004 kg, $P = 0.01$ and 0.856 ± 0.005 kg, $P < 0.025$ respectively) than T0 pullets (0.631 ± 0.005 kg and 0.831 ± 0.003 kg). No differences in U or CV were observed on d51 or d72 ($P > 0.05$). On d73, T0 had a longer latency to right from TI (213.7 ± 28.852 s, $P = 0.001$) than T1 (111.4 ± 13.152 s), T3 (113.5 ± 13.892 s), and T4 (144.1 ± 19.991 s). Pullets fed dietary Trp had a shorter latency to right from TI on d53 and d73 (128.33 ± 9.209 s, $P < 0.02$ and 142.03 ± 9.736 s, $P = 0.003$, respectively) than T0 (182.1 ± 25.893 s, and 213.7 ± 28.852 s, respectively). Pullets fed dietary Trp also spent less time in the second phase of TI on d53 and d73 (54.76 ± 6.629 s, $P = 0.003$ and 101.27 ± 8.223 s, $P < 0.05$, respectively) than T0 (109.20 ± 23.986 s and 142.40 ± 23.871 s, respectively). A shorter latency to first head movement during TI was observed on d73 in pullets fed dietary Trp (40.76 ± 4.748 s, $P = 0.019$) compared to T0 (71.3 ± 17.656 s). No differences by treatment or feed were found in INV intensity (flaps/s, $P > 0.05$). These results indicate dietary inclusion of supplemental Trp promotes pullet welfare and improves the efficiency of BW gain while maintaining strain-specific performance guidelines.

Key Words: pullet, nutrition, welfare, tryptophan, fear

320P Differences in expression of frolicking and running behavior in conventional and slow-growing strains of broiler chickens. Lucas J. Zilli*^{GS 1}, Lauren Dawson¹, Zhenzhen Liu³, Ruth Newberry², Stephanie Torrey¹, Tina Widowski¹; ¹*Animal Biosciences, University of Guelph, Guelph, Ontario, Canada*, ²*Norwegian University of Life Sciences, Ås, Norway*, ³*University of Guelph, Guelph, Ontario, Canada*.

Play behavior is a positive welfare indicator that may influence the debate concerning the use of conventional versus slower-growing strains of broiler chickens. Accordingly, the objective of this study was to determine if play behavior differed between broiler strains differing in growth rate. Strains of slower-growing (SG) broilers were divided into 3 categories based on growth rate: 4 FAST

($ADG_{0-61} = 53.5-55.5$ g/d), 4 MOD ($ADG_{0-61} = 50.2-51.2$ g/d) and 4 SLOW ($ADG_{0-61} = 43.6-47.7$ g/d) and compared to 2 strains of conventional (CONV; $ADG_{0-47} = 66.0-69.7$ g/d). Over 8 trials, strains were segregated by pen and blocked by location within the experimental room. In total, data was collected from 11 pens for CONV, 58 pens for FAST, 60 pens for MOD and 65 pens for SLOW. A free space method was used to stimulate play at 35 d of age for CONV, and 35 and 49 d of age for SG. For this, birds were moved to the back of the pen using a cardboard divider, and the feeder and hanging scale were removed from the pen, creating an empty “free space” in their pens; each pen was then video recorded for 5 min. All instances of frolicking (wing-assisted running, jumping, and wing flapping) and running (running and wing-assisted running) performed by individually-marked focal birds were recorded using Observer software. For analysis, behaviors were categorized into a binary outcome, whether or not behaviors occurred in any of the focal birds. Generalized linear mixed models with repeated measures were run using SAS® to compare odds ratios of flocking and running behavior between all categories at 35 d and across two different ages, 35 and 49 d, within the SG categories. At 35 d, SLOW, MOD and FAST strains were 7.82 ($p = 0.0116$), 6.70 ($p = 0.0203$), and 5.02 ($p = 0.0407$) times more likely to engage in frolicking behavior, respectively, compared to CONV. At 35 d, running only differed in SLOW birds, who were 5.60 times more likely to perform the behavior ($p = 0.0207$) than CONV. In contrasts statements across SG categories, running was more likely to occur in MOD compared to FAST at 49 days ($p = 0.0309$). When comparing age differences within category, running declined with increasing age in SLOW ($p = 0.0210$) and FAST ($p = 0.0443$), but not in MOD birds ($p = 0.5263$). These results demonstrate that play behavior is expressed more by slower-growing broiler strains compared to conventional strains when matched for age. This suggests that there may be factors, such as physical limitations, that impede the ability of conventional broilers to express play, or that prevent them from reaching their individual motivational thresholds to perform play behaviors.

Key Words: Broiler, Welfare , Play, Behavior, Slow-growing

321P Impact of pen density on environmental enrichments usage in broiler chickens. Annie Lozano*^{GS}, Katy Tarrant; *Animal Science and Agricultural Education, Fresno State, Fresno, California, United States*.

Environmental enrichment use in a broiler house is one strategy to increase environment complexity, encourage physical activity, and promote natural behaviors. Vertical integrators are continuously evaluating novel methods, such as enrichments, to be used to promote animal welfare. As a low-cost animal protein option, it is particularly important to conventional producers to understand the impact of including environmental enrichments in facilities, as it relates to increased production costs. Therefore, studies

evaluating the efficacy of enrichments, and their implementation in commercial facilities, is warranted. In an effort to better understand the minimum number of enrichments that are needed in a facility to balance animal well-being and cost effectiveness, we evaluated bird usage of platform-style enrichments in a commercial barn. Eight mini-pens encapsulating a water line, and equipped with two tunnel feeders, were constructed in a 1,486m² tunnel ventilated house. Each mini pen contained one rectangular platform with a usable surface area of 0.27m². Two replicates of pens each contained 5, 10, 15, and 20 broilers each (P5, P10, P15, P20). Bird usage was recorded using cameras installed in the ceiling of the house. Observations were made at five time points. Total bird count and bird frequency (number of birds on the platform / number of birds in the pen) was collected from d2 to d43 of growout. All five time points were averaged per day, and a Tukey's HSD was used to separate means. Data was evaluated using fit least squares in JMP v.14. Average usage over the entire flock for P5, P10, P15, and P20 was 0.74, 1.68, 2.24, and

3.13 birds, respectively. Despite having the same usable platform space amongst pens, P5 had lower usage than P20 pen throughout the flock (0.60 ± 0.4 versus 2.8 ± 0.4 on d6 ($P= 0.01$); (1.8 ± 0.5 versus 4.4 ± 0.5 on d43 ($P= 0.01$)). Significant differences of the two main effects, total bird count and age, were found in both replicates ($P < 0.0001$). The interaction of the two main effects was also significant in both replicates ($P= 0.02$). While pens varied in bird density in this study, usable enrichment area remained the same. We would expect, given all other variables are the equal, that birds would occupy the platforms at the same rate, regardless of density. Interestingly, this was not the case. For this study, we aimed to determine if broiler usage of platform enrichments varies based on bird density as a starting point in determining the minimum number of this type of enrichments needed to achieve both high welfare standards, and low costs. Further study is needed in order to understand how preference and hierarchy may play a role in broiler usage of these platforms.

Key Words: broiler, enrichment, platform

Genetics and Molecular Biology

322P Exosomes derived from lipopolysaccharide-stimulated chicken macrophages regulate immune response through the MyD88/NF- κ B signaling pathway.

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Exosomes from immune cells can regulate immune responses of recipient cells by releasing their contents such as mRNAs, microRNAs, and proteins molecules. In the immune system, macrophages recognize lipopolysaccharides (LPSs) of gram-negative bacteria by toll-like receptor 4 (TLR4) and intracellular pathways, such as NF- κ B pathway, are activated, inducing pro-inflammatory cytokine expression. The purpose of this study was to investigate the immunoregulatory functions of LPS-stimulated exosomes in chicken immune systems. Therefore, chicken macrophages cells (HD11) were stimulated with μ g/mL LPS from *Escherichia coli* O127:B8, and exosomes were purified. The purified exosomes were characterized by size and exosomal marker. The immunomodulatory effects of LPS-activated exosomes were evaluated by qRT-PCR and western blotting. Statistical analyses were performed using IBM SPSS software. Results with a p-value of <0.05 were considered statistically significant. Differences between groups were evaluated using Tukey's multiple comparison test. In a qRT-PCR result, the LPS-activated exosomes regulated the gene expression of cytokines and chemokines, including IL-1 β , IFN- γ , IFN- α , IL-4, CCL4, CCL17, and CCL19, in naive chicken macrophages. Furthermore, in a western blotting result, LPS-stimulated exosomes induced the MyD88/NF- κ B signaling pathway through MyD88, TAK1, and NF- κ B1 molecules. Taken together, as an immune response against gram-negative bacterial infection, LPS-stimulated chicken macrophages can release exosomes that are delivered to inactivated macrophages by regulating the expression of immune-related genes and the MyD88/NF- κ B signaling pathway.

Key Words: exosomes, lipopolysaccharide, NF- κ B signaling pathway, chicken, macrophage

323P In vitro validation of newly constructed chicken oviduct gene promoters using luciferase assay.

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A chicken as a bioreactor has been widely used to produce human therapeutic proteins, and exhibited a meaningful performance owing to approval from the Food and Drug Administration in 2015. Several types of chicken ovalbumin promoters have been developed, a promoter reconstructed

with 2.8-kb ovalbumin promoter (pOV) and 1.6-kb ovalbumin estrogen response element (ERE), was considered to have potent activity and tissue specificity. Since, cargo size of a viral vector is limited, compact and efficiently functional promoter is required to drive transgene expression in chicken. Here, we newly developed two types of chicken oviduct gene promoters, putative enhancer elements derived from other chicken oviduct genes, such as ovotransferrin (TF), ovomucin alpha subunit (OVOA), ovalbumin-related protein X (OVALX) were linked to a 5'-flanking region of the pOV (TF_pOV, OVOA_pOV, OVALX_pOV) as first type promoters. Also, the promoters derived from other chicken oviduct genes, such as ovotransferrin (pTF), lysozyme (pLYZ), ovomucoid (pOVM) were linked to a 3'-flanking region of the ERE (ERE_pTF, ERE_pLYZ, ERE_pOVM) as second type promoters. These six recombinant promoters were cloned into luciferase assay vector (pGL4.11), transfected into human and chicken somatic cells as well as, primarily cultured chicken oviduct cells, and validated with relative luciferase activity. The relative luciferase ratio was analyzed using oneway ANOVA. The recombinant promoters, TF_pOV, OVOA_pOV in the first and ERE_pLYZ, ERE_pOVM in the second type promoters had 2.1- to 19.5-fold ($p < 0.05$) higher luciferase activity than a reconstructed ovalbumin promoter (ERE_pOV). In conclusion, the promoters constructed with ovalbumin and other chicken oviduct genes can be utilized to efficiently drive a transgene expression in chicken oviduct.

Key Words: chicken oviduct genes, ovalbumin, recombinant promoters, oviduct cells, luciferase assay

324P Neuropeptide Y and its receptors are expressed in chicken skeletal muscle and regulate mitochondrial function. Elizabeth S. Greene*¹, Ahmed Dhamad¹, Marco Zampiga², Federico Sirri², Sami Dridi¹; ¹*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*, ²*Agricultural and Food Sciences, Alma Mater Studiorum - University of Bologna, Bologna, Italy.*

Neuropeptide Y (NPY) is a highly conserved neurotransmitter which is primarily expressed in the hypothalamus. It is a potent orexigenic neuropeptide, stimulating appetite and inducing feed intake in a variety of species. Recent research has shown that NPY and its receptors can be expressed by peripheral tissues, but their role is not well defined, particularly in avian species. Therefore, the aim of this study was to determine the expression of NPY and its receptors and determine their regulation by environmental and nutritional stressors in skeletal muscle of avian species via in vivo and in vitro approaches. 32 day-old Cobb 500 chicks were divided into 4 weight-matched groups and reared for 8d with ad libitum access to water and feed. On day 9, birds received an intraperitoneal injection of recombinant rat NPY (rNPY: 0, 60, 120 or 240 ng/100g BW). Feed intake was recorded

hourly, and 3 hours after injection, birds were humanely euthanized by cervical dislocation. Breast and leg muscle samples were immediately collected, frozen in liquid nitrogen, and stored at -80°C for further gene expression analysis. For in vitro experiments, QM7 cells were fasted overnight then treated with 100nM rNPY for 24 h, with untreated cells used as control. Cells were then subjected to gene expression analysis via real-time PCR or cellular bioenergetics via Seahorse XFp Analyzer. Data were analyzed by Student's T-test or one-way ANOVA, as appropriate. Significant differences among individual group means were determined with Student-Newman-Keuls multiple range test. NPY and its receptors are expressed in chicken breast and leg muscle as well as in the QM7 cell line. Intraperitoneal injection of recombinant NPY increased feed intake in 9d old chicks and upregulated the expression of NPY and NPY receptors in breast and leg muscle, suggesting autocrine and/or paracrine roles for NPY. Additionally, NPY is able to modulate the mitochondrial network. In breast muscle, a low dose of NPY upregulated ($P < 0.05$) the expression of genes involved in ATP production (UCP, NRF2) and dynamics (MFN1), while a high dose decreased ($P < 0.05$) markers of mitochondrial dynamics (MFN1, OPA) and increased ($P < 0.05$) genes involved in mitochondrial biogenesis (D-loop, PPAR γ). In leg muscle, NPY decreased ($P < 0.05$) markers of mitochondrial biogenesis and ATP synthesis (D-loop, PCG1 α , PCG1 β , PPAR γ , NRF2). In QM7 cells, genes associated with mitochondrial biogenesis, dynamics, and ATP syntheses were all upregulated ($P < 0.05$), even though basal respiration and ATP production were decreased ($P < 0.05$) with NPY treatment. Together, these data show that the NPY system is expressed in avian skeletal muscle and plays a role in mitochondrial function.

Key Words: NPY, skeletal muscle, mitochondria

325P Seasonal and sexual variation in mRNA expression of selected adipokine genes affecting fat deposition and metabolism of Emu (*Dromaius novaehollandiae*). Ji Eun Kim^{*1}, Darin C. Bennett^{1, 3}, Kristina Wright², Kimberly M. Cheng¹; ¹Avian Research Centre, University of British Columbia, Vancouver, British Columbia, Canada, ²Canada's Michael Smith Genome Sciences Centre, University of British Columbia, Vancouver, British Columbia, Canada, ³Animal Science Department, California Polytechnic State University, San Luis Obispo, California, United States.

Emus are farmed for the production of oil rendered from their back and abdominal fat. Emu oil has good anti-oxidant and anti-inflammatory properties and can promote cell growth. Adult emus start to gain fat in spring and summer and utilize the energy from the accumulated fat to sustain themselves during breeding in winter as they have little feed intake during that time. Objective is to examine the mRNA expression of 7 adipokine genes that are known to affect fat deposition and metabolism in birds to identify gene markers that could help improve emu fat production. We tracked 11

adult emus (7 males, 4 females) over a breeding season. Back and abdominal fat tissues were biopsied in April, June, August and November. In November, the birds were slaughtered and BW and fat pads weights were recorded. Total RNA was isolated from the adipose tissue and cDNA was synthesized. Gene specific primers were designed and used for partial cloning fragments to amplify the open reading frame of *FABP-4* (Fatty acid binding protein), *SCD-1* (Stearoyl-CoA desaturase), *AdipoQ* (Adiponectin), *AdipoR1* (Adiponectin Receptor 1), *AdipoR2* (Adiponectin Receptor 2), *Lept* (Leptin), *LepR* (Leptin Receptor) and β -*actin* (House-keeping gene). mRNA expression of each gene was assessed by quantitative RT-PCR and differential expression analysed by the $2^{-\Delta\Delta C_T}$ method. Seasonal expression variation was analysed by LSANOVA and association of fat gain (FG) and expression level was by multiple regression. *eFABP4* expression was high in April and November. FG from April (Ap) to August (Au) regressed positively on Apr and June (Ju) expression, respectively. FG from Au to November (Nv) regressed negatively on Au expression. Female *eSCD-1* expression in Au was 35 X higher than other months. FG from Ju to Au regressed positively on Ju expression. *eAdipoQ* expression was highest in Ap and lowest in Nv. FG Ju to Au regressed positively on Ju expression and FG from Au to Nv regressed negatively on Au expression. *eAdipoR1* expression was highest in Ap and lowest in Ju. FG from Ap to Ju regressed negatively on Ap expression. FG from Ju to Au regressed negatively on June expression. *eAdipoR2* expression was highest in Ap and lowest in Nv. FG from Au to Nv regressed positively on Ju expression. No *Lept* expression in emu adipose tissue. *eLepR* expression was highest in Ap and lowest in Nv. FG from Ju to Au regressed negatively on Ju expression. There has been no selection study for increasing fat deposition in farm animals. Our study laid down the groundwork for identifying promising candidate genes for such purpose. More studies into the multiple-genetic factors are needed to develop novel molecular markers that can be applied to improve fat production in emus.

Key Words: emu, fat deposition, adipokine genes

326P Microbiota diversity in duodenum, jejunum and ileum of emu (*Dromaius novaehollandiae*). Ji Eun Kim^{*1}, Hein M. Tun³, Darin C. Bennett^{1, 2}, Dengwei Zhang³, Kimberly M. Cheng¹; ¹Avian Research Centre, University of British Columbia, Vancouver, British Columbia, Canada, ²Animal Science Department, California Polytechnic State University, San Luis Obispo, California, United States, ³HKU-Pasteur Research Pole, School of Public Health, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong, SAR, China.

Emus are farmed for the production of oil, which is rendered from their back and abdominal fat pads. Emu oil has good anti-oxidant and anti-inflammatory properties and has ingredients that promote cell growth. While fatty acid composition of the oil was proposed to cause its antioxidant

properties, the effective ingredients in emu oil that may confer therapeutic benefits remain undefined. Objective is to characterize the intraluminal intestinal microbiota in the small intestine segments (duodenum, jejunum, and ileum) and examine the metabolic functions of emu gut bacteria to see whether they may contribute to the effective ingredients in emu oil. Small intestine segments were collected from 4 adult emus and the contents were scraped from the intestinal wall of each segment. Genomic DNA was extracted from each of the four duodenal, jejunal and ileal samples and the variable region 3 to 5 (V3-V5) of the bacterial 16S rRNA gene amplified by PCR and sequenced by 454 GS Junior. The cleaned sequences were clustered into OTUs. Taxonomy was assigned to each OTU. Representative sequences of each OTU were aligned and the hypervariable regions were filtered. PICRUSt was used to predict functional genes of the classified members of the microbiota. Welch's t-test used for the predicted microbiota functions. PICRUSt nMDS plot showed that the predicted metagenomics functions of the cecum are very different from that of the small intestine. Duodenum: higher vitaminB2 metabolism than ceca. Jejunum: higher toxin degradation, fatty acids biosynthesis and lipid metabolism than ceca, higher protein metabolism than duodenum and ceca, higher antibiotics biosynthesis than duodenum. Ileum: higher toxin degradation and vitaminA1 metabolism than ceca, higher protein metabolism than duodenum. Ceca: higher sugar metabolism, protein digestion and absorption, antibiotics biosynthesis and antigen presentation than duodenum, jejunum and ileum, higher vitaminB6 metabolism than jejunum and ileum, higher carbohydrate metabolism and absorption than jejunum, higher lipid degradation than duodenum. Our analysis indicated that both the jejunum and ileum high toxin degradation function which reflects the free-range condition of the emu. The jejunum has fatty acids biosynthesis and lipid metabolism function which may result from natural selection for the emu to accumulate large amount of fat for energy storage. The ileum may share fibre digestion with the ceca. Further research in relationship between microbial metabolites and fat composition is worthwhile.

Key Words: emu, gut microbiota, metagenomic functions

327P No presentation materials submitted.

328P A 127 Kilobase Deletion affecting the Carboxy Peptidase Q gene in broilers. Duaa A. Almansaf*; *Biological Department, University of Arkansas, Fayetteville, Arkansas, United States.*

Ascites syndromes is a pathophysiological condition resulting from body demand for oxygen in fast-growing broilers. The result is increased pressure within the pulmonary circulation leading to accumulation of fluid in the abdominal and then mortality. We used Whole Genome Resequencing (WGR) to identify genetic and chromosomal regions related to ascites. This identified 31 chromosomal regions of genes associated with ascites. One of these

regions is a 127 kbp region on chromosome 2 encompassing the 3' end of the gene for Carboxypeptidase Q (CPQ; also known as Plasma Glutamate Carboxypeptidase). CPQ encodes a metallopeptidase, in the family of M29 peptidases, that plays a significant role in the cleavage of dipeptides into amino acids. Based on next generation sequencing data, some broilers carry a deletion of this region beginning in intron 4 of the CPQ gene to ~36 kbp downstream of exon 8. We developed PCR primers that span the deletion allowing us to fine map the exact limits of the deletion which appears to have resulted from recombination of two CR1 repeat. We have used exonuclease PCR assays to genotype for relative levels of exon 2 and 8 to quantify the prevalence of the deletion in our REL ascites research line. This line represents an unselected closed population derived from a commercial broilerelite line from the 1990s. Further work is focused on the contributions of this deletion to the affect of the CPQ region on ascites phenotype.

Key Words: CPQ gene, Ascites

329P No presentation materials submitted.

330P Profiling and functional analysis of Insulin-induced circular RNAs in pectoralis muscle of different chicken breeds. Binghao Shao*^{GS1}, Lin.Ge Gao², Xinghao Zhu³, H.Y Zhang¹, W Chen¹, Y.Q. Huang²; ¹*Henan Agricultural University, ZhengZhou, China,* ²*Henan Agricultural University, ZhengZhou, China,* ³ *Henan Agriculture University, Henan Province, China.*

To investigate the insulin-responsive circRNAs in different breeds of chicken skeletal muscle, 24d old male Arbor Acres broilers were injected with insulin (RI), and PBS as a control (RP), respectively. Each chicken in the treatment group was intraperitoneally injected with insulin of 5IU/kg, while the control group was injected with the same dose of PBS. The pectoralis muscles of 3 broilers in each group were collected for RNA-seq. Similarly, RNA-seq was performed in silky fowls at 120min after injection (WI120, WP120). A total of 2027 circRNAs were identified. We found that the number of differentially expressed circRNAs in WP120_vs_RP120 was the most, with 64 up-regulated and 62 down-regulated, which may be related to chicken breeds. The difference of circRNAs in RI15_vs_RP15 was the least, indicating that circRNAs response to insulin in broilers were relatively less at 15min. Enrichment analysis showed that the source gene was mainly involved in insulin secretion, amino acid synthesis, glucose metabolism, and MAPK signal pathway. We predicted the target miRNAs of circRNAs by miRanda, and selected | log₂FC | top 10 circRNAs from the results list to construct a circRNA-miRNA interaction network. Some miRNAs (miR-103, miR-107, and miR-26a) that bind to circRNAs are reported to be involved in the regulation of insulin metabolism. There are also miRNAs (miR-124, miR-146b, and miR-181) related to muscle growth and development. Eight circRNAs were randomly selected and qRT-PCR was used

to confirm the reliability of RNA-seq. A circRNA (circINSR) formed by exons of insulin receptor gene (*INSR*) was screened from sequencing data. Four silky chickens of different ages (E10, E19, 24d, 49d) were taken from each group and qRT-PCR was used to investigate the expression characteristics of circINSR in different tissues. One-way the analysis of variance (ANOVA) showed that the expression of circINSR in pectoralis muscle changed greatly, and the relative expression of circINSR in the pectoralis muscle of silky fowls at 24d was higher than that in the heart, liver, muscle stomach, glandular stomach and leg muscle ($P < 0.05$). $P < 0.05$ was considered significant. We also explored the effects of food restriction on circINSR. The results showed that the expression of circINSR in the pectoralis muscle of broilers after 15% energy restriction was lower than that of the control group ($P < 0.05$); while the relative expression did not change significantly after 15% protein restriction. In summary, we obtained circRNAs produced by different varieties of chicken pectoralis muscle after insulin injection, which provided a reference for further study on the regulatory mechanism of circRNAs in insulin in chicken skeletal muscle.

Key Words: circRNA, exogenous insulin, pectoralis muscle, chicken

331P Identification and functional analysis of insulin-responsive lncRNA in chicken skeletal muscle. Ziyang Wang*^{GS}, Pengfei Du, Xiangli Zhang, Yongshuai Wang, H.Y Zhang, Y.Q. Huang, W Chen; *College of Animal Science and Technology, Henan Agricultural University, ZhengZhou, Henan, China.*

To obtain the key genes of insulin-responsive in broilers, this study was conducted to explore the effect of exogenous insulin on the expression of lncRNA in chicken pectoralis. Twenty 24-day-old broilers were randomly divided into two groups with 10 birds per treatment and received with insulin intraperitoneally (5 IU/kg) or phosphate buffered saline (PBS) after 16 h of fasting. Blood glucose were determined, and pectoralis were collected for strand-specific RNA sequencing. Differentially expressed lncRNAs (DELs) target mRNA was predicted and analyzed with the bioinformatics analysis method. The outcome of two-tailed unpaired t-test showed that broilers (Arbor Acres) had the lowest blood glucose in 120 min after insulin injection, and the blood glucose in the broiler treated by exogenous insulin was significantly lower than that in the PBS group ($P < 0.01$). Regarding the analysis and identification of DELs by EdgeR ($Q < 0.05$; |Fold Change| ≥ 2), 48 up-regulated and 27 down-regulated DELs were detected in pectoralis. Among them, glucose homeostasis, mitogen-activated protein kinase activity (MAPK), muscle development, amino acid activity, insulin secretion were enriched through GO analysis. Insulin signal pathway, melanin production, glycolysis and gluconeogenesis, mTOR signal pathway, MAPK signal pathway were identified by KEGG pathway. In addition, 7 DELs 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 sequencing results, indicating that the sequencing results were reliable. Collectively, this study provides the prediction and analysis of lncRNA function in the process of chicken skeletal muscle insulin signal transduction, and these results provide a theoretical basis for follow-up research.

Key Words: insulin, broiler, skeletal muscle, lncRNA

332P Effects of exogenous insulin and energy restriction on PPP1R3C expression in arbor acres broilers. Lin.Ge Gao*^{GS}¹, Binghao Shao², Xinghao Zhu³, H.Y Zhang¹, Y.Q. Huang¹, W Chen¹; ¹Henan Agricultural University, ZhengZhou, China, ²Henan Agricultural University, ZhengZhou, China, ³Henan Agriculture University, Henan Province, China.

There has been no systematic study on PPP1R3C (protein phosphatase 1 regulatory subunit 3C) in poultry. The purpose of this study was to detect the expression of PPP1R3C in different tissues of arbor acres broilers and to explore the effects of exogenous insulin and energy restriction on the expression of PPP1R3C in insulin-sensitive tissues of broilers. In experiment 1: male broilers were intraperitoneally injected with insulin (5IU/kg) or PBS at d 24. Tissue samples were taken at different time points (0 min, 15 min, 120 min and 240 min, n=5) after injection. The expression of PPP1R3C in different tissues at 0min after injection and at different time points after injection were detected by qPCR; In experiment 2: Female broilers of d 48 were randomly selected and divided into two groups. The control group (energy 13.17MJ/kg, crude protein 19%) was free access to conventional diet, while the energy restriction group was subjected to 30% energy restriction and fed with 80% ration of the average daily feed intake of the control group. The expression of PPP1R3C in breast muscle and liver was detected at d 48; In experiment 3: Female broilers at d 7 were randomly divided into three groups. The control group (energy 12.97 MJ/kg, crude protein 36.45%) was fed with conventional diet; The energy restricted group was fed with energy restricted diet (with 15% energy restriction); The protein-restricted group was fed a protein-restricted diet (15% protein restriction), and all broilers had free access to diets. The expression of PPP1R3C in breast muscle was detected after feeding to d 21. Data were analyzed by one-way ANOVA, and significance was set at $P < 0.05$. 1) PPP1R3C was predominantly expressed in breast muscle, followed by heart and leg muscle. 2) The expression of PPP1R3C did not significantly change after PBS injection ($P > 0.05$). After insulin injection, the expression of PPP1R3C was decreased in the breast muscle and liver ($P < 0.05$), while the expression of PPP1R3C in abdominal fat increased at 15min and then recovered to 0min level at 240min ($P < 0.05$). 3) The expression of PPP1R3C in breast muscle and liver was significantly down-regulated by 30% energy restriction

($P < 0.05$). 4) 15% energy restriction and 15% protein restriction did not significantly affect the expression of *PPP1R3C* in breast muscle ($P > 0.05$). The above studies indicate that *PPP1R3C* is predominantly expressed in breast muscle, while insulin injection can reduce the expression of *PPP1R3C* in breast muscle and liver, and compensatively increase the expression of *PPP1R3C* in abdominal fat. 30% energy restriction could produce an effect similar to exogenous insulin, and the effect of energy restriction on *PPP1R3C* mRNA level was dose-dependent.

Key Words: PPP1R3C, chicken, energy restriction, insulin

333P Seasonal and sex dependent gene expression in emu (*Dromaius novaehollandiae*) fat. Kristina Wright*^{GS}¹, Ka Ming Nip¹, Ji Eun Kim², Kimberly M. Cheng², Inanc Biro¹; ¹Canada's Michael Smith Genome Sciences Centre, University of British Columbia, Vancouver, British Columbia, Canada, ²Avian Research Centre, University of British Columbia, Vancouver, British Columbia, Canada.

Emu (*Dromaius novaehollandiae*) is a bird farmed for its oil, rendered from fat, for uses in therapeutics and cosmetics. Emu oil has anti-inflammatory and antioxidant properties, promoting wound healing. Emus go through an annual cycle of fat gain and loss where Canadian emus, used in this study, start gaining fat in April, continue gaining fat in June, and start losing fat in November during breeding season. This study seeks to determine what genes affect emu fat metabolism and deposition using transcriptome analysis as part of an aim to improve emu fat production. Back and abdominal fat tissues of the same 4 male and 4 female emus were collected in April, June, and November. mRNA samples from fat tissues were isolated to perform next-generation sequencing (NGS). In November, the birds were slaughtered and body and fat pad weights were recorded. NGS reads were analyzed to find out what genes are differentially expressed (DE) depending on different seasons and sex and what key genes are involved in fat metabolism. Transcriptome from emu fat tissue was measured with Illumina MiSeq sequencer and quantified with Salmon. Fat expression profiles were annotated with Ensembl emu transcriptome and analyzed for differential expression in DESeq2 for 12 pair-wise comparisons between three time points for both and each of the sexes, and between the sexes at each of the three time points. Gene ontology (GO) terms and enriched pathways for seasonally DE genes were compared between the sexes using gprofiler2. Seasonally DE genes functioning in multiple pathways were examined. Differential expression analysis utilizes MLE and pathway enrichment was analysed by ORA. We found 100 DE genes (47 seasonally in males, 34 seasonally in females, 19 between sexes) and of those 79 annotated DE genes (35 seasonally in males, 27 seasonally in females, 17 between sexes). Seasonally DE genes generating significant difference between the sexes in GO terms as well as supporting studies suggested ITGB2 (Integrin beta chain-2) influences fat changes. 6 seasonally DE genes functioned in >2 pathways (2 female: ANGPTL4

(Angiopoietin-like 4) and LPL (Lipoprotein lipase); 4 male: LUM (Lumican), OGN (Osteoglycin), ALDOB (Aldolase B), and SLC37A2 (Solute Carrier Family 37 Member 2)). Two sexually DE genes (FSHR (Follicle Stimulating Hormone Receptor) and PLIN2 (Adipose differentiation-related protein)) had functional investigations supporting their influence on fat gain and loss. The results and related studies suggested 9 key genes (ITGB2, ANGPTL4, LPL, LUM, OGN, ALDOB, SLC37A2, FSHR, PLIN2) that functionally influence fat metabolism and deposition in emus. These genes should be further looked at in future studies of emu fat production.

Key Words: Emu, Differential Expression, Fat Deposition

334P Detection of differentially expressed genes in broiler *Pectoralis major* muscle affected by spaghetti meat and woody breast. Sunoh Che*^{GS}¹, Chaoyue Wang², Shai Barbut², Csaba Varga³, Christian Fuchs⁴, Phuc H. Pham¹, Dorothee Bienzle¹, Leonardo Susta¹; ¹Pathobiology, University of Guelph, Guelph, Ontario, Canada, ²Food Science, University of Guelph, Guelph, Ontario, Canada, ³Pathobiology, University of Illinois, Urbana, Illinois, United States, ⁴Maple Leaf Foods, Mississauga, Ontario, Canada.

Spaghetti meat (SM) and woody breast (WB) are myopathies characterized respectively by firm consistency (fibrosis) and marked separation of myofibers, which occur in fillets of high breast-yield and growth-rate broilers. These conditions lead to economic losses due to reduced consumer acceptance and poor meat quality. Transcriptomic studies have suggested that the pathogenesis of WB may be associated with muscular hypoxia and oxidative stress; however, no evaluation of the transcriptomic changes in SM-affected fillets has been documented. Therefore, the goal of the study was to investigate the transcriptomic profiles of SM- and WB-affected chicken fillets, to outline a framework of the gene networks associated with these myopathies. The *Pectoralis major* muscle from 32 male broilers (39 d, Ross 708) was collected from a processing plant immediately after exsanguination, and scored for the presence of WB and SM. Total RNA was extracted, and samples with RNA integrity numbers > 7 were used for cDNA synthesis. Eighteen samples that passed the quality test (normal = 6; SM = 7; WB = 5) were used for library preparation and normalized for pair-end sequencing. The significance threshold for differentially expressed genes was set at a false discovery rate of < 0.1 and a minimum fold change of 1.5. The principal component plot and the heatmap cluster analysis showed that WB samples clustered together and separated from normal or SM samples, whereas normal and SM samples clustered together. A total of 1,315 genes were differentially expressed between SM- and WB-affected fillets, with 459 genes upregulated and 856 genes downregulated in the SM-affected group. There was no significant difference in expressed genes between normal samples and SM-affected fillets. When comparing both the WB vs. normal, and SM vs. WB groups, 1) Gene

Ontology (GO) analysis showed significant enrichment of terms associated with the extracellular environment and the immune response; 2) the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis showed three significantly enriched pathways, including focal adhesion, phagosome, and extracellular matrix-receptor interactions. These findings show that WB has a transcriptomic profile that differs from both SM-affected and normal fillets, indicating that WB is a condition associated with the regulation of genes involved in interactions between cells and the extracellular environment and the immune response. On the contrary, lack of significant differences in expressed genes between the SM-affected and normal fillets suggests that SM may not be caused by factors intrinsic to the broiler breast muscle, and could be caused by mechanical disruptions at the processing plant (e.g., plucking).

Key Words: broiler myopathy, pathway analysis, RNA sequencing, spaghetti meat, woody breast

335P Antimicrobial resistance and phylogenetic groups in *Escherichia coli* isolated from broiler chickens in the State of Paraná, Brazil. Mikaela D. Adur*^{GS 1, 2}, Jean-Yves Madec², Marisa Haenni², Renata Macedo¹; ¹*Pontifical Catholic University of Paraná, Curitiba, Brazil*, ²*National Agency for Food Safety, Environment and Occupational Health, Lyon, France*.

Escherichia coli are commensal bacteria present in both animals and humans. Antimicrobial use in poultry production has been described as a source of antibiotic

resistance. In certain cases, *E. coli* from poultry can be directly transmitted to humans but also pathogenic and resistance mechanisms can be passed on to other bacteria that colonize the human host, which are then able to cause disease. This study aimed to assess the antimicrobial resistance in *E. coli* isolated from broiler chickens in the state of Paraná, Brazil. In total, 160 cloacal swabs were collected from 27 to 49-day old broilers from eight different poultry farms belonging to five different companies in the state of Paraná. The extended-spectrum β -lactamase (ESBL) phenotype was characterized by double-disc synergy and AmpC producing by reducing sensibility of ceftiofur disc. The DNA of each isolate was extracted using the boil method. *E. coli* strains were classified using PCR with specific primers for each phylogenetic group (A, B1, B2 and D). Descriptive statistical data analyses were performed. Bacteria isolates were found in 100% (160/160) of cloacal samples. Resistance phenotypes were identified, of which 32.5% (52/160) were ESBL, 7.5% (12/160) AmpC and 0.6% (1) carried both phenotypes. In addition, all phylogenetic groups were identified, of which 17% (27/160) A, 46% (74/160) B1, 7% (12/160) B2 and 25% (41/160) D. This study demonstrated a high prevalence of antimicrobial resistance phenotypes in *E. coli* isolates, especially ESBL phenotype, that is responsible for the global spread of *E. coli* infections, that is one of the greatest threats to human and animal health.

Key Words: Poultry, resistance, ESBL, AmpC, Phylogroup

Immunology, Health and Disease

336P Development and characterization of poultry-specific immune reagents and immunoassays: NIFA grant progress report. Hyun Lillehoj*; ARS, USDA, Beltsville, Maryland, United States.

There are two main challenges in poultry immunology and poultry disease research: 1) the lack of poultry-specific immunological reagents since mammalian reagents do not recognize poultry proteins, and 2) the lack of methods to effectively measure host immune response to many poultry infectious diseases. NIFA-supported systematic plan to address the poultry immunological reagent gap with the long-term goal to develop critical immunological tools for poultry species began in 2012 as part of the US Veterinary Immune Reagent Network (VIRN). This report will give the progress of what has been accomplished in the developing and commercializing chicken reagents including 25 genes encoding cytokines and chemokines and the production of mouse monoclonal antibodies detecting major chicken cytokines and chemokines in biological samples using antigen-capture ELISA. Most immune reagents (60 hybridomas and 14 chicken cytokines/chemokines) which we developed under the NIFA-funded grants have been widely distributed through commercialization. Availability of these poultry immune reagents will facilitate fundamental and applied research in poultry diseases and enable the development of vaccines and antibiotic alternative feed additives for poultry researchers and scientists who conduct comparative immunology.

Key Words: immunity, immune reagents, cytokines, chemokines, antigen capture assays

337P Tissue colonization in broiler chickens after oral challenge with *Salmonella* Heidelberg field strains. Clarissa S. Vaz*, Daiane Voss-Rech, Francisco N. da Fonseca, Marcos A. Zanella Morés, Arlei Coldebella; *Embrapa Suínos e Aves, Concordia, Santa Catarina, Brazil.*

A high prevalence of *Salmonella* Heidelberg has been reported in broiler farms in southern Brazil, reflecting an increasing number of carcasses contaminated at slaughter. This study aimed to determine the potential tissue colonization of *S. Heidelberg* field strains in broilers. Two *S. Heidelberg* strains (BRM 42033 and BRM 53672) isolated from broiler houses in southern Brazil were selected based on different antimicrobial resistance and *Xba*I-pulsed-field gel electrophoresis patterns. Day-old SPF White Leghorn chicks were inoculated with 10^5 CFU of BRM 42033 or 10^6 CFU of BRM 53672 by oral gavage, separated in two experimental groups according to each strain, and housed in air-filtered wire-mesh floor isolator chambers. Feed and water were provided *ad libitum*, and mortality and clinical signs of diseases were daily monitored. Between 10 to 12 broilers from each group were randomly selected, euthanized and necropsied at days 5, 12, 19 and 26 after challenge, and tissues were aseptically

removed for *Salmonella* analyses. Cecal content was subjected to quantitative analysis in brilliant green agar supplemented with 40 mg/mL of novobiocin and 30 mg/mL of streptomycin + 15 mg/mL of gentamycin for BRM 42033 or 50 mg/mL of nalidixic acid for BRM 53672 for selective plate counting. Qualitative analysis was carried out in liver, spleen and cecal tonsils. *Salmonella* \log_{10} CFU/g measured in cecal content at each necropsy was subjected to an analysis of variance pondered by the inverse of the variance of each *S. Heidelberg* strain for the model containing the effects of strain, days after challenge, and the interaction strains x days. Fisher exact test was used to compare qualitative results for each strain on each necropsy. Mortality, detected from days 2 up to 8 after challenge, was higher in chicks challenged with BRM 42033 (10/52, 19.2%) than BRM 53672 (2/50, 4%). BRM 42033 showed higher cecal colonization in all evaluated days compared with BRM 53672 ($p \leq 0.05$). At 26 days after challenge, strain BRM 42033 and BRM 53672 showed *S. Heidelberg* levels of 5.08 ± 1.06 and $2.10 \pm 2.42 \log_{10}$ CFU/g in the cecal content, respectively. Strain BRM 42033 also showed higher number of positive spleens at days 5, 12 and 19; livers at days 5 and 12; and cecal tonsils at days 12, 19 and 26 after challenge ($p \leq 0.05$). Although strains showed differences in colonization potential, the effective colonization of tissues by *S. Heidelberg* highlights broilers as potential sources of contamination at slaughter and the need for control measures at farms.

Key Words: salmonellosis, poultry, food safety

338P Anisomorphism – a case of poikilocytosis or a marker of subtle toxicity in heterophils? Paul Cotter*; Biology, Framingham State University, Arlington, Massachusetts, United States.

Description of the problem: the avian heterophil is regarded as an integral component of innate immunity. Because it is phagocytic, capable of degranulation, produces reactive oxygen species, and releases a myriad of antimicrobial substances it is an effective defense against invasive microbes. Presumably the antimicrobial capacity of heterophils depends on an undegraded physiologically intact cell. When this is not the case defenses are likely compromised. Certain changes of heterophils recognized microscopically as toxicity are the presence of vacuoles, increased cytoplasmic basophilia, abnormal granulation (dark blue to purple granules) granules with abnormal shapes, and abnormal staining. Here the objective is to describe atypical heterophils of ducks displaying changes suggestive of functional degradation. Materials and methods: atypical cells were detected in blood films from ducks of various ages, stained with Wright-Giemsa, examined at 45x magnification, and photographed at 100x. The results show that shapes of some cells could range from hexagonal to higher order polyhedrons. The descriptive term “anisomorphism” is used for this type of

poikilocytosis. Anisomorphic cells could display fully stained nuclei and cytoplasmic granules. Alternately irregularly shaped cells may have a poorly stained (hypochromic) nucleus or cytoplasm. Such heterophils were detected in the blood of ducklings as early as day 1 and in older ducks of both sexes. Anisomorphic cells are not technical artifacts or distorted because of pressure from neighboring cells. Nor is anisomorphism a consequence of sample staleness as blood films from day 1 ducklings were direct smears not exposed to EDTA or other anticoagulants. Conclusions: most heterophils are produced in bone marrow where the developmental stages must pass through the lining cells of sinus walls (diapedesis). Circulating heterophils must again pass through the linings of blood vessels in order to reach sites of inflammation. Presumably both processes require some cell membrane flexibility and the normal spherical shapes of heterophils must change temporarily. However, the shape distortions of anisomorphic heterophils appear to occur during the circulatory phase. This suggests abnormality. The cause is unknown but given the importance of heterophils in defense, anisomorphism is remarkable type of poikilocytosis and is suggested to represent a subtle form of toxicity.

Key Words: duck, heterophil, atypia, blood, toxicity

339P Comparison of immune cell composition and metabolic responses in peripheral blood mononuclear cells of three inbred chicken genetic lines during *Eimeria* challenge. Krysten Fries-Craft*, Susan Lamont, Elizabeth A. Bobeck; *Department of Animal Science, Iowa State University, Ames, Iowa, United States.*

Coccidiosis due to *Eimeria spp.* is an economically damaging disease in both layer and broiler production. Iowa State University maintains diverse, highly inbred lines with divergent immune responses to pathogen challenge. Differential cytokine expression between resistant and susceptible genetic lines has been described, but alterations in immune response are not well-documented. The objective was to compare immune cell population and metabolic phenotype responses to *Eimeria* challenge between Leghorn (Ghs6 and Ghs13) and Fayoumi (M5.1) genetic lines. Sixty birds/line were placed in battery cages at hatch (10 birds/cage) and fed a commercial diet. On d21, 10 birds/line were euthanized for peripheral blood mononuclear cell (PBMC) isolation. Half of the remainder were orally inoculated with 10X Merck Coccivac®-B52 vaccine (Kenilworth, NJ) resulting in 6 total study groups. Blood from 5 birds/group was collected on 1, 3, 7, and 10d post-inoculation (pi). Agilent real-time ATP and glycolytic rate assays (Santa Clara, CA) were used to determine oxidative and glycolytic contributions to ATP production and glycolytic rate, respectively, as indicators of immune cell activation. Monocyte/macrophage and lymphocyte population changes during *Eimeria* challenge were assessed by multicolor flow cytometry. Data were analyzed using the MIXED procedure (SAS 9.4) with fixed effects of genetic line, *Eimeria* challenge, and line × challenge, and

significance at $P \leq 0.05$. Prior to inoculation, M5.1 birds had greater monocyte/macrophage⁺, Bu-1⁺ B cell, and CD3⁺ T cell populations compared to both Ghs lines ($P < 0.0001$) but similar immunometabolic phenotype. *Eimeria*-challenged M5.1 birds had 34.5% increased peripheral blood monocytes/macrophages compared to unchallenged counterparts on 1dpi ($P = 0.003$). In contrast, a significant 54.3% monocyte/macrophage increase in Ghs6 birds did not occur until 7dpi ($P = 0.006$). On 10dpi, both Ghs6 and Ghs13 birds had 46.4-49.8% fewer CD3⁺T cells than non-challenged controls ($P = 0.002$). No differences in total CD3⁺ T cells between challenged and non-challenged M5.1 birds were observed at 10dpi. Despite early immune cell population responses, metabolic profiles were only different at 10dpi when *Eimeria*-inoculated Ghs6 and Ghs13 birds had a 24.0-31.8% increase in glycolytic contributions to overall ATP production compared to non-challenged counterparts ($P = 0.04$). These results suggest M5.1 birds have a faster systemic monocyte/macrophage response without altering immunometabolism during *Eimeria* challenge, while Ghs lines responses may rely primarily on T cell recruitment from peripheral blood in conjunction with increased metabolic indicators of immune activation in later stages post-inoculation.

Key Words: *Eimeria*, Genetic Line, Flow Cytometry, Immunometabolism, Immunity

340P *Clostridium perfringens* enumeration and toxin expression in a model with *Eimeria maxima* and influences in mineral digestibility after each component of the challenge. Matthew K. Jones*¹, Jennie Baxter¹, Anita Menconi³, Charles L. Hofacre¹, Roy Berghaus²; ¹*Southern Poultry Research Group, Inc., Watkinsville, Georgia, United States*, ²*Department of Population Health, University of Georgia, Athens, Georgia, United States*, ³*Evonik Corporation, Kennesaw, Georgia, United States.*

As consumer preferences encourage the poultry industry to use less antibiotics, limiting both *Clostridium* and *Eimeria* interventions, NE is increasingly difficult to manage. Understanding the relationship between these pathogens and the host may aid in disease intervention. In the current study, ileal mineral digestibility, excreta *Clostridium sp.* enumeration, and toxin expression were evaluated at different times during a necrotic enteritis challenge. Six replicate cages of 10 male Ross broilers represented each of two treatment groups: a challenged group and an unchallenged group. The challenged group received *Eimeria maxima* oocysts (~5000/bird) at 14 days of age and were gavaged with 1.0 mL of a field CP isolate (1×10^8 CFU/mL) on day 20. Ileal content was collected from 4 birds/pen and pooled to evaluate mineral digestibility on day 20 and 23. CP was enumerated and analyzed by qPCR for toxin expression on day 14, 20, 21, 22 and 23. ANOVA, linear mixed, and Tobit censored regression models were applied to digestibility, CP enumeration, and toxin expression data, respectively.

Some minerals had lower digestibility coefficients in the challenged group on day 20, specifically sodium, potassium, and molybdenum ($P < 0.05$). By day 23, mineral digestibility had largely recovered; however, nickel digestibility was higher in the challenged group ($P < 0.05$). On days 21 and 22, CP populations were greater in the challenged group ($P < 0.05$). Enumeration of *Clostridium* via direct or enriched culture was not different between challenged and unchallenged groups at other time points. Temporally, enumeration data suggested greater populations of *Clostridium sp.* in the excreta at 14 days which waned prior to CP challenge. Cpa toxin was present in both groups prior to CP challenge but had increased abundance in the challenged group from day 20 to day 23 ($P < 0.05$). NetB was not detected in either group prior to the challenge. After the CP gavage, NetB expression was higher in the challenged group ($P < 0.05$). While sodium and potassium disturbances have been previously reported in mixed *Eimeria* challenges, this data suggests that *E. maxima* is a major contributor to this secretion. Further investigation is needed to understand differences in molybdenum digestibility on day 20 and nickel uptake in the challenged controls on day 23. While direct enumeration, cpa toxin, and NetB toxin expression all successfully differentiated the challenged group in relation to an unchallenged control, NetB toxin may be the easiest to interpret in an applied situation due to less expression in non-pathogenic commensal CP populations.

Key Words: Necrotic enteritis, *E. maxima*, mineral digestibility, cpa toxin, NetB toxin

341P Saponins-citroflavonoids based solution reduced by 70% the growth of *Histomonas meleagridis*: an *in vitro* study. Mohammed El Amine Benarbia*^{1, 2}, Pierre Chicoteau^{1, 2}; ¹R&D, Nor Feed, Angers, France, ²Labcom FeedInTech, Beaucauzé, France.

Histomonas meleagridis is a flagellate protozoan parasite living in the caecum of the digestive system of birds. It is the causative agent of histomonosis or commonly known as “Blackhead disease” in turkey. While chicken are asymptomatic carriers of the parasite, the effect on turkey is more dramatic. Indeed, the mortality due to this infestation can reach 80 to 100% leading to important economic losses. Feed supplementation with plant-based solutions can be considered as a part of a globale management for modern turkey producers. This study was performed in order to assess the *in vitro* the effect of active compounds of Citronin XO (CXO), a saponin-citroflavonoids based solution developed by Nor Feed SAS; France, on the growth of *H. meleagridis* and compare their effects to metronidazole. 100,000 *H. meleagridis* cells grown in log phase were added to Dwyer’s media with the compound to be tested and incubated at 40°C for 48 hours. Metronidazole a 12.5 ppm was used as a positive control, while media was used as a negative control. CXO solution was applied at 500 ppm. Counts was performed using a Neubauer hemocytometer. 3 replications per treatment and each replicate was counted 4

times. Statistical analysis was performed by one-way ANOVA test using Graph pad Prism software. Statistical significance was considered at $p < 0.05$. Results show that Both metronidazole and CXO reduced the growth of *histomonas meleagridis* *in vitro*. As expected, incubation with metronidazole reduced the growth by 99.65% ($P < 0.001$) compared to control. Active compounds of CXO, namely saponins and citroflavonoids, were able to reduce the growth by 70% compared to control after incubation with the parasite ($P < 0.05$). Results from this *in vitro* study showed that saponins and citroflavonoids active compounds of CXO reduced the growth of the causative agent of blackhead disease by 70% in these experimental conditions. These results support the fact that feed supplementation with CXO could contribute to improve turkeys’ gut health and reduce antimicrobial use in livestock. *In vivo* studies with and/or without parasite challenge are needed to confirm this *in vitro* results.

Key Words: Saponins, Turkey, *Histomonas meleagridis*, Gut health, Citrus extract

342P *Salmonella* chitosan nanoparticle vaccine administration is protective against *Salmonella* Enteritidis in broiler birds. Keila Y. Acevedo-Villanueva*^{GS 1}, Sankar Renu², Renukaradhya Gourapura², Ramesh Selvaraj¹; ¹Poultry Science, University of Georgia, Athens, Georgia, United States, ²Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio, United States.

Salmonella poultry vaccines have a withdrawal period of twenty-one days before slaughter. Live vaccines require two doses, three to four weeks apart, whereas inactivated vaccines can be administered simultaneously or after live vaccines. Administering a live vaccine followed by a killed vaccine booster can eliminate the time restrictions of live vaccine boosters and allow producers to comply with the withdrawal period. We evaluated the efficacy of administering a live *Salmonella* vaccine followed by a killed *Salmonella* chitosan nanoparticle (CNP) booster vaccine on broilers. The live *Salmonella* vaccine used was Poulvac®ST. The CNP vaccine was synthesized with *Salmonella* Enteritidis (SE) outer-membrane-proteins (OMPs) and flagellin proteins. At d1-of-age, one-hundred-sixty-eight chicks were allocated into treatments: 1) No vaccine, 2) Poulvac®ST vaccine, 3) CNP vaccine, 4) Poulvac®ST+CNP vaccine. Chicks were allocated in 6 pens/treatment with 7 chicks/pen. At d1, birds were orally vaccinated with PBS, Poulvac®ST, or CNP. At d7, birds were orally vaccinated with PBS or CNP. At d14, birds were orally challenged with 1×10^9 CFU/bird SE. At d7, d14, 8h-post-challenge, d21, and d28, anti-OMPs IgY and IgA were analyzed in serum and cloacal swabs. At 8h-post-challenge, d21, and d28, anti-OMPs IgA were analyzed in bile. At d28, SE loads in the ceca and IL-1 β , IL-10, IFN- γ , and iNOS mRNA from cecal tonsils were analyzed. Body-weight-gain (BWG) and feed-conversion-ratio (FCR) were recorded weekly. Data were analyzed by One-way ANOVA at

$P < 0.05$. There were no significant differences in BWG or FCR between vaccinated birds compared to control. At d14, Poulvac[®]ST+CNP-vaccinated birds had 46% greater levels ($P < 0.05$) of anti-OMPs IgY in serum, compared to control. At 8h-post-challenge, CNP and Poulvac[®]ST+CNP-vaccinated birds had 17% and 24% greater levels ($P < 0.05$) of anti-OMPs IgA in bile, respectively, compared to control. At 1wk-post-challenge, CNP and Poulvac[®]ST+CNP-vaccinated birds had 17% and 24% greater levels ($P < 0.05$) of anti-OMPs IgA in bile, respectively, compared to control. At 2wk-post-challenge, CNP, Poulvac[®]ST, and Poulvac[®]ST+CNP-vaccinated birds had 33%, 18%, and 24% greater levels ($P < 0.05$) of anti-OMPs IgA in bile, respectively, compared to control. At d28, CNP and Poulvac[®]ST+CNP-vaccinated birds had 0.9 Log₁₀ CFU/g and 1 Log₁₀ CFU/g decreased SE cecal loads ($P < 0.05$), respectively, compared to control. There were no significant differences in IL-1 β , IL-10, IFN- γ , and iNOS mRNA between vaccinated birds compared to control. Findings demonstrate that the CNP prime vaccination and CNP booster vaccination can induce an antigen-specific immune response against SE and can decrease SE cecal load on broilers.

Key Words: Salmonella, Nanoparticles, Vaccines, Chitosan, Broilers

343P valuation of anti-mycotoxin additives in poultry intestinal explants challenged with aflatoxin. Cristina T. Simões*^{GS}, Vinícius Duarte, Daniel F. Soares, Diogo Liberalesso, Cristiane R. da Silva, Luara M. Schlösser, Giséle P. da Rosa, Carlos A. Mallmann; *Department of Preventive Veterinary, Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil.*

In vitro tests are performed to evaluate the efficacy of anti-mycotoxin additives (AMAs) in preventing mycotoxicosis in livestock. Such tests do not present high correlation with *in vivo* trials, which are currently required by the legislation to certify AMAs efficiency. In search of an alternative method, a study was conducted to investigate the utilization of an *ex vivo* technique to evaluate different AMAs in their efficiency to reduce intestinal absorption of aflatoxin B1 (AFB₁) in poultry. Six AMAs with three different compositions were evaluated: AMA1 and AMA2 from hydrated sodium calcium aluminosilicates (HSCAS); AMA3, AMA4 and AMA5 from HSCAS + yeast cell wall (YCW); and AMA6 from HSCAS + YCW + seaweed extracts. A total of 60 Cobb 500 male broilers were allocated in battery cages and fed a common diet free of aflatoxins, fumonisins, zearalenone, deoxynivalenol, and T-2 toxin contamination (verified via high performance liquid chromatography tandem mass spectrometry). At 24 days, birds were slaughtered and 4 jejunal explants were collected from each bird (n=240) and immediately fixed in the Ussing Chamber. Six different trials were conducted, each trial had two treatments (The control treatment: Buffer solution (BS) + 2.8 mg/L of AFB₁ and the AMA treatment: BS + 2.8 mg/L of AFB₁ + 0.5% of AMA) with 20 replicates distributed in

a completely randomized block design with each bird being considered as one block. Additionally, AMAs were tested *in vitro* to assess AFB₁ adsorption in artificial intestinal fluid (pH = 6). Statistical analyses were conducted using the Statgraphics Centurion XV software. Mean differences in AFB₁ adsorption among the AMAs were separated using Tukey's test. Pearson's correlation was performed between *ex vivo* and *in vitro* results. In the *ex vivo* tests, all AMAs reduced ($P < 0.05$) the AFB₁ concentration in jejunal explants when compared to the Control treatment. The AMA1 and AMA2 reduced the AFB₁ jejunal absorption by 67.67% and 74.24%, respectively, whereas AMA3, AMA4, AMA5 and AMA6 presented more expressive reduction of AFB₁ intestinal absorption, with 80.31%, 85.79%, 86.28% and 82.30%, respectively ($P < 0.05$). No differences were observed among AMAs efficiency in the *in vitro* test ($P > 0.05$), with all the adsorptions being higher than 99%. No correlation ($P > 0.05$) was observed between *ex vivo* and *in vitro* results. The *ex vivo* model demonstrated to be a useful tool in the assessment of anti-mycotoxin additives efficiency for poultry. Further studies are needed to better understand the correlation between *ex vivo* and *in vivo* results in order to decrease the number of birds in practical experiments.

Key Words: Aflatoxin B1, anti-mycotoxin additive, broiler, intestinal explant, Ussing chamber

344P Adaptation of cell culture assay measuring fluorescent quantification of β -D-Glucuronidase activity for assessment of ileal granulocyte degranulation in tissue scrapings. Audrey F. Duff*^{GS}, Kaylin Chasser, Kate McGovern, Michael Trombetta, Lisa Bielke; *Animal Sciences, The Ohio State University, Wooster, Ohio, United States.*

Inflammation is a complex innate immune response that protects mucosal tissues against excessive damage or antigens and facilitates return to homeostasis. Uncontrolled gastrointestinal (GIT) inflammation can cause dysregulation of GIT homeostasis, decreased gut barrier integrity, and increased disease risk. Methods that assess localized inflammation and innate immune response would lend insight into mechanisms by which GIT conditions affect bird health. Heterophil granule component β -D-glucuronidase has been used to assess degranulation activity in cell culture supernatant after bacterial challenge. Adaptation of this assay for site specific degranulation in GIT tissue was assessed as a potential indicator of GIT inflammation. Further, IL-8 plays a role in granulocyte recruitment and could be assessed in tandem to supplement degranulation measurements via ELISA. Three replicate experiments were conducted to evaluate the ability to detect GIT granulocyte degranulation after *in ovo* inoculation with saline (S), or 10² CFU of *Citrobacter freundii* (C) or lactic acid bacteria (L). Inoculations were administered on embryonic d18, and samples collected from 6 birds/treatment on day of hatch (DOH; Exp.1) and d10 included ileal scrapings for degranulation and IL-8 (Exp.2

and 3). Body weight gain (BWG) was measured in all experiments. Degranulation samples were collected into RPMI (1% penicillin/streptomycin) media on ice and centrifuged to collect supernatants that were tested in 3 replicates and incubated with 4-methylumbelliferyl- β -D-glucuronide for 4h at 45°C. Liberated 4-methylumbelliferone (4-MU) was quantified fluorometrically at an excitation/emission wavelength of 360nm/460nm. Treatments were compared against S via Dunnett's test. No significant differences were seen in Exp.1. In Exp.2, C 4-MU was significant at $p=0.068$ while IL-8 was $p=0.6218$. There were no 4-MU differences in Exp.3 ($p>0.05$), while L IL-8 had a p -value of 0.069. In all experiments, BWG was insignificant which suggests inflammation may not have been high enough to measure differences with 4-MU and IL-8. While IL-8 was hypothesized to mirror degranulation, these results may suggest the need of a different substantiating marker, or that IL-8 recruitment was not a major contributing factor to degranulation in GIT tissue. Further, high standard errors within the 4-MU assay support that a strong unidirectional inflammatory response may be needed for separation of treatment effects. While adaptation of the 4-MU assay to tissue extracts was not confirmed, this may be due to a lack of observed inflammation, as indicated by insignificant BWG. Nonetheless, this assay has potential to serve as

Key Words: gastrointestinal inflammation, degranulation, heterophil, in ovo, IL-8

345P Effect of probiotics on early microbial colonization in day of hatch ducklings. Michael Trombetta*^{GS 1}, Kaylin Chasser², Audrey F. Duff³, Kate McGovern³, Denise Russi Rodrigues³, Debbie Jeffery⁵, Daniel J. Shafer⁵, Lisa Bielke⁴; ¹*Animal Science, The Ohio State University, Wooster, Ohio, United States*, ²*Animal Sciences, Ohio State University, Columbus, Ohio, United States*, ³*Animal Sciences, The Ohio State University, Wooster, Ohio, United States*, ⁴*OSU, Wooster, Ohio, United States*, ⁵*Maple Leaf Farms, Leesburg, Indiana, United States*.

Early microbial colonization of the gut can influence health and performance throughout life. Lactic acid-producing bacteria (LAB) have been suggested to positively influence gastrointestinal microbial populations and overall health. Embryos from poor performing and good performing parent flocks were hatched without treatment (C) or sprayed in the hatching cabinet at d18 of embryogenesis with a mixed inoculum LAB probiotic of *Lactococcus salivarius* and *Pediococcus parvulus* (P). Some results were previously presented, and this report investigated the overall treatment impact on microbiome regardless of parent source to determine probiotic effect on pioneer colonizers. On DoH, intestines were aseptically collected for microbial analysis via Illumina next-generation sequencing. All relative abundances were compared against C using Student's T-test. In Exp.1, Phyla Bacteroidetes, Tenericutes, Spirochaetes, Verrucomicrobia, and Fibrobacteres were increased

($p<0.05$) by application of the probiotic. At family level, Lactobacillaceae, Fibrobacteraceae, Bacillaceae, and Bifidobacteriaceae were among those increased ($p<0.05$) in P. This is positive as Lactobacillaceae and Bifidobacteriaceae are considered integral components of beneficial microbial development. Additionally, a decrease in Enterobacteriaceae in P was seen that trended towards significance ($p=0.0605$) which is beneficial as Enterobacteriaceae tend to include opportunistic pathogens. In Exp.2, Phyla Bacteroidetes, Spirochaetes, Fibrobacteres, Tenericutes, TM7, and Verrucomicrobia were increased ($p<0.05$) in P. At family level, Lachnospiraceae, Ruminococcaceae, and Fibrobacteriaceae were among those increased ($p<0.05$) in P. In addition, Streptococcaceae ($p=0.0297$) was reduced and Enterobacteriaceae trended downward ($p=0.0742$) towards significance in P. Many species within Streptococcaceae and Enterobacteriaceae can cause opportunistic infections in birds and reduction is a marker of beneficial microbial development. Based on changes observed in the microbiota following application of P, it is fair to say that a significant shift has taken place. Based on an increase of beneficial gut bacteria such as Bifidobacteriaceae, Lactobacillaceae, and Fibrobacteriaceae, the importance of early microbial population of the gut and the effect of probiotics has been displayed. These families are shown to cause positive changes in inflammation, intestinal permeability, and overall bird performance which is why their increase is significant. The decrease in potentially pathogenic families such as Streptococcaceae and Enterobacteriaceae is regarded as an additional positive outcome due to a lower risk of disease incidence and replacement by beneficial bacteria.

Key Words: Microbiome, Probiotic, Hatchery, Lactic Acid-Producing Bacteria, Ducks

346P Entero-V Poultry, a botanical liquid blend, limits coccidiosis impact in *Eimeria*-vaccinated broilers. Jonathan Pierron*^{GS 1, 2}, Bertrand MEDINA³, Marie-Christine Frenette⁴, Simon Cloutier⁴, Ivan D. Girard³, Carl Julien^{1, 5}; ¹*Centre de Recherche en Sciences Animales de Deschambault (CRSAD), Deschambault, Quebec, Canada*, ²*Microbiology, biochemistry and bioinformatics, Université Laval, Quebec, Quebec, Canada*, ³*Probiotech International Inc., St-Hyacinthe, Quebec, Canada*, ⁴*Triple-V Ambulatory Veterinary Services, Acton Vale, Quebec, Canada*, ⁵*Animal Sciences, Université Laval, Quebec, Quebec, Canada*.

Coccidiosis vaccines are widely used on day-old chicks, but their benefit: risk ratio is not always clear on fast-growing broiler flocks. Since the last decades, botanical extracts have been employed to encourage reasoned use or withdrawal of antimicrobial drugs. This study evaluated the impact of a proprietary botanical liquid blend in combination with *Eimeria* vaccination in coccidiosis-challenged broilers. A total of 1,485 Ross 308 male broiler chickens were allocated to three treatments: non-infected

(NI) birds; *Eimeria*-infected (EI) birds; EI birds + 0.25 mL/L Entero-V Poultry (EV, Probiotech) in drinking water for 10 consecutive days (d14-d24). All birds were vaccinated with IMMUCox3® (CEVA) at the hatchery. The same 3-phase diets were offered *ad libitum* to 9 replicates of 55 birds from 0 to 35 days of age. No antibiotics nor anticoccidials were used. *Eimeria* challenge was induced by an oral administration at d14 of 2×10^5 sporulated oocysts of mixed *Eimeria* species harvested from field isolates. Body weights (BW) and feed intakes were recorded on day 10, 20 and 35 and reported per period, as mortality rates. Performance data and fecal 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). Mortality rates did not differ significantly between treatments. Despite vaccination, EI showed, at 6 days post-infection (6 dpi, d20), a trend in reducing BW (734 vs 780 g, $P=0.1025$), worsened feed conversion ratio (FCR) during the grower period (10-20d) (1.50 vs 1.37, $P=0.0254$), at 6 dpi *Eimeria* spp. excretion peak (18.2 vs 3.5×10^5 oocyst per gram (OPG), $P<0.0001$) and 7 dpi intestinal *E. tenella* lesion score (1.33 vs 0.22, $P=0.0002$), compared to NI birds. EV increased BW (6 dpi, d20) (793 vs 734 g, $P=0.0429$) and improved FCR during the starter period (0-10d) (1.18 vs 1.23, $P=0.0113$), the grower period (10-20d) (1.33 vs 1.50, $P=0.0058$) and overall (0-35d) (1.29 vs 1.33, $P=0.0160$), compared to EI birds. EV also reduced 6 dpi *Eimeria* spp. excretion (11.4 vs 18.2×10^5 OPG, $P<0.0001$) and decreased 7 dpi intestinal *E. tenella* lesion score (0.61 vs 1.33, $P=0.0076$), compared to EI birds. Overall, EV limited negative impacts of coccidiosis outbreak in *Eimeria*-vaccinated broilers by improving growth performance and showing an anticoccidial activity, especially against *E. tenella*. This tested botanical liquid blend can be introduced on top off any coccidiosis control strategies based on vaccination and without anticoccidials, to secure broiler performances.

Key Words: *E. tenella*, ABF production, intestinal health, botanical extracts, broilers

347P Measuring *Eimeria* oocysts viability via auto-fluorescence following anticoccidial treatment. Kate McGovern*^{GS1}, Johel Bielke¹, Audrey F. Duff¹, Alamanda Calvert², Kaylin Chasser¹, Lisa Bielke¹; ¹The Ohio State University, Wooster, Ohio, United States, ²BioMatrix Intl, Princeton, Minnesota, United States.

Eimeria is a major cause of disease within the poultry industries and anticoccidial resistance makes control a challenge and highlights the need for development of new control technologies. In vitro tests against oocyst stages can allow for anticoccidial activity of candidate prophylactics in an affordable and timely manner. Three experiments were conducted to determine how anticoccidial candidates (AC) would affect oocyst viability as determined by oocyst auto-fluorescence, morphology assessment, and excystation to

verify microscopic assays. Exp.1 consisted of 5 treatments including a 45°C control (45C) and AC1-AC4. Treatments in Exp. 2 and 3 included a roomtemperature control (RTC), 45C, and AC5. In all experiments, each treatment had 5 replicate tubes consisting of *Eimeria tenella* oocysts and corresponding AC or PBS in RTC and 45C tubes. Treatment and 45C were incubated on shakers at 45°C while RTC remained at room temperature. In Exp. 1, subsamples of each replicate were collected for microscopic evaluation at 72h incubation and in Exp. 2 and 3 subsamples were imaged at 48h incubation. Photomicrographs consisted of a FITC filtered image overlaid onto a brightfield image of the same field of view. Sporulated, non-fluorescent oocysts were counted as infective while non-sporulated, abnormal, or fluorescent oocysts were counted as non-infective. In Exp. 2 and 3, excystation was performed once AC1 images showed ~80% non-infective oocysts to determine sporozoite motility and thus potential infectivity. All viability counts were analyzed by chi-square in SAS with significance denoted at $p<0.05$. In Exp. 1 all treatments showed a significant decrease in oocyst infectivity relative to 45C. Similarly, both Exp. 2 and 3 showed a significant increase of non-infective oocysts for AC5 compared to RTC and 45C. In Exp. 2, motile sporozoites were observed in RTC and 45C treatments while non-motile sporozoites were observed in the AC5 treatment. In Exp. 3, RTC and 45C contained excysted sporozoites, while no sporozoites were observed in AC5 but, many sporocysts still contained non-excysted sporozoites suggesting they were no longer viable. Based on these results, probable oocyst infectivity was observed by oocyst auto-fluorescence, morphology and decreased sporozoite excystation. The final excystation step confirmed that fluorescence and abnormal morphology served as indicators of decreased infective potential, making the assay a potential tool for in vitro screening of candidate anticoccidial compounds that act on oocyst stages of *Eimeria* before in vivo testing.

Key Words: *Eimeria*, drug resistance, anticoccidial, excystation, fluorescence

348P Evaluation of quinine as a prophylactic candidate against *Histomonas meleagridis*. Lesleigh C. Beer*^{GS}, Billy M. Hargis, Christine Vuong; *Poultry Science, University of Arkansas: Division of Agriculture, Fayetteville, Arkansas, United States.*

Histomoniasis, also commonly referred to as blackhead disease, is caused by the protozoan parasite *Histomonas meleagridis*. Since the removal of nitarsone (Histostat) in late 2015, there are no approved prophylactics available for mitigating histomoniasis. Disease incidence and high mortalities are frequently associated with turkey flocks, although infection of broiler breeders also occurs. Quinine is a naturally occurring alkaloid with antimalarial properties. *In vitro* assays have shown strong antihistomonal properties of quinine, leading to our hypothesis that quinine inclusion within the feed could prevent histomoniasis in turkeys. Selected concentrations of

quinine were included within a turkey starter diet to evaluate effects on body weight gain (BWG), liver lesions, cecal lesions, and mortality of *Histomonas meleagridis*-challenged turkeys. On day-of-hatch, poultts were randomly assigned to either the basal diet or a quinine diet. Groups consisted of a non-challenged control (NC; basal diet), 0.022% quinine + challenge, 0.067% quinine + challenge, 0.2% quinine + challenge, or a positive-challenged control (PC; basal diet). On d10, challenged groups were intracloacally inoculated with 1×10^5 *H. meleagridis* cells/turkey, and lesions were evaluated on d21 post-infection. Individual body weights were recorded on d0, d10, and d31 to calculate the pre-challenge and post-challenge BWG. No significant differences ($P > 0.05$) were observed between the d0-10 pre-challenged BWG between quinine treatment diets and the basal diet. Similarly, no differences ($P > 0.05$) were observed in post-challenge d10-31 BWG of the quinine dietary treatments as compared to the PC. Cumulative mortalities, liver lesions, and cecal lesions related to histomoniasis were not reduced ($P > 0.05$) in any of the quinine treatment groups as compared to the PC. Although quinine successfully reduced *H. meleagridis* cells *in vitro*, results from the *in vivo* experiment indicated no reduction in histomoniasis severity, as evidenced by similar lesions and mortality as the PC. Taken together, these data indicate that quinine inclusion within the feed at these concentrations and under these experimental conditions was not efficacious in the prevention or treatment of histomoniasis.

Key Words: histomoniasis, *Histomonas meleagridis*, quinine, turkey, blackhead

349P Effect of Clostridium septicum hemolytic activity units on Clostridial dermatitis (cellulitis) bacterin/toxoid humoral immunogenicity in turkeys. Aaron Forga*^{GS 1}, Danielle Graham², Makenly Coles¹, Lesleigh C. Beer³, Callie Selby¹, Lucas Graham¹, Roberto S. Cuesta¹, Jared Ruff¹, Guillermo Tellez-Isaias¹, Billy M. Hargis¹, Christine Vuong¹; ¹*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*, ²*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*, ³*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*.

Clostridium septicum (CS), a toxigenic, anaerobic spore-former, is the primary etiology responsible for cellulitis/dermatitis in commercial turkeys. The alpha toxin is the predominant virulence factor associated with CS pathogenesis. In commercial turkeys, vaccination with a CS bacterin-toxoid oil emulsion vaccine reduced penicillin usage and CS-associated mortality under field conditions. During production of CS antigen, hemolytic activity assays have been traditionally utilized to determine toxin antigen load prior to formalin inactivation. However, the hemolytic assay is not specific to alpha toxin activity because the delta toxin produced by CS is also hemolytic. In the present study, multiple batches (50L each) of CS antigen were prepared with a range of hemolytic activity ranging from

128 to 1024 hemolytic units (HU) at the time of formalin inactivation to compare antigen HU to resulting immune response post-vaccination. For each antigen batch produced: pH, cell density, cell morphology, and hemolytic activity at selected time points during incubation were evaluated. Immunized treatment groups (N=20 per treatment) included: 1) non-immunized control (NC), 2) 128 HU antigen + mixing incubation, 3) 512 HU antigen + mixing, 4) 1024 HU antigen + mixing, 5) 128 HU antigen + static incubation, 6) 1 part 64 HU and 1 part 1024 HU antigen batches + mixing, or 7) 5 parts 64 HU and 1 part 512 HU antigen batch + mixing. Commercial turkeys were transferred from an integrator to our facilities at 6 weeks-of-age and immunized subcutaneously at the nape of the neck (0.5mL/turkey) at 7 weeks-of-age. Blood was collected weekly from 7 to 12 weeks-of-age to determine serum antibody response to CS alpha-toxin post-vaccination. From 9 to 12 weeks-of-age, group 2 presented with significantly ($P < 0.0001$) higher antibody titers to CS alpha-toxin, as measured by ELISA, compared to the NC and the other 5 vaccinated groups. Similar results were observed for group 5 from 10 to 12-weeks-of-age. Group 4 had the lowest antibody titers throughout the duration of the study, although the antigen had the highest reported hemolytic activity (1024 HU) before formalin inactivation. These data suggest that multiple factors contribute to CS alpha-toxin production/bioactivity and must be considered when estimating CS alpha-toxin presence with a hemolytic activity assay in lieu of minimum lethal dosage testing. **Keywords:** dermatitis, cellulitis, turkeys, *Clostridium septicum*, vaccine

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

350P Effect of oral or intracloacal challenge with Escherichia coli or Salmonella Enteritidis on enteric colonization and early performance in broiler chickens. Callie Selby*^{GS 1}, Danielle Graham², Lucas Graham¹, Aaron Forga³, Mikayla Baxter¹, Guillermo Tellez-Isaias¹, Billy M. Hargis¹, Christine Vuong¹; ¹*Poultry Science, University of Arkansas, Bella Vista, Arkansas, United States*, ²*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*, ³*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*.

Escherichia coli (*E. coli*) and *Salmonella* Enteritidis are common pathogens introduced to neonatal poultry. Both pathogens can be vertically and horizontally transmitted during oviposition, in the hatching environment, and at the broiler farm. Colonization of these bacteria can have substantial implications for the poultry industry due to reduced flock performance, condemnations in the processing plant, and possible zoonosis. In a laboratory setting, *E. coli* and *Salmonella* challenges are commonly administered via oral gavage at day-of-hatch. Little research has been conducted to evaluate the efficacy of challenging chicks with *Enterobacteriaceae* via intracloacal gavage.

The objectives of these studies were to evaluate 1) the efficacy of an intracloacal versus oral gavage challenge at day-of-hatch and 2) dose for optimal colonization utilizing either *E. coli* or *S. Enteritidis*. In Exp 1, day-of-hatch chicks were randomized, weighed, and challenged via oral or intracloacal gavage with saline (vehicle), *E. coli*, or *S. Enteritidis* at selected doses (10^3 , 10^4 , or 10^5 CFU/0.25mL/chick). Based upon Exp 1, significant differences ($P<0.05$) in GIT colonization and body weight at days 3 and 7, final challenge doses of 10^4 CFU *S. Enteritidis* and 10^5 CFU *E. coli* were selected for administration via cloacal or oral gavage in Exp 2. Body weight gain and colonization of the gastrointestinal tract were evaluated on days 3, 7, and 14 of Exp 2. On day 3, significantly more *S. Enteritidis* ($P<0.001$) were

enumerated in the challenged groups than the non-challenged control, but differences were not observed when comparing the two administration methods. No differences for Gram-negative recovery were observed between the non-challenged control and *E. coli* challenged groups at 72 hours post-challenge. In contrast to previous reports indicating that intracloacal challenge induced increased colonization with salmonellae, this was not observed in the present experiments. Similarly, the presence of presumptive *E. coli* colonization in challenged and non-challenged chicks suggests that this approach as a challenge model, at least with non-pathogenic challenge models, may not be successful.

Key Words: *Escherichia coli*, *Salmonella*, broiler, challenge, model

Management and Production

351P Evaluation of a functional sensory feed additive on broiler performance, blood chemistry and carcass yield under heat stress conditions. Jean-François Gabarrou*³, Carlos T. Gonzales³, Aurélie Auvray, Eduardo Bernal¹, William Narváez-Solarte²; ¹*Spin Colombia SAS, Bogota, Colombia*, ²*Universidad Caldas, Manizales, Colombia*, ³*Phodé, Terssac, France*.

The neurosensory anti-stress effect of citrus extract (CE) additive via an olfactory-serotonergic systems was evaluated and proved on mice. Thus, this specific citrus extract was investigated on growth performance, blood chemistry and carcass yield on broiler under heat stress conditions during 21 days. 320 chicks (1-day-old Ross 308) were housed vertical cages of 0.36 m² (20/cage) in two rooms. Broilers were splitted into 2 treatments: (VeO) the citrus extract group (VeO, Laboratoires PHODE, France - 250 g/MT of feed); (CTL) the negative control. Both rooms were kept at a constant temperature and relative humidity of 38±1°C and 60%rH. Difference between groups were analysed by Tukey test. At 21 days, (Na+K-Cl) blood content was more concentrated from CTL birds (n=5) than VeO group (n=5) (41.09 vs 35.36 mmol/L, P<0.05) as same as blood protein content (35.69 vs 30.81 g/L, P<0.05) indicating a higher dehydration status for CTL birds. Thus, broilers receiving VeO maintained their feedintake compared to CTL ones (812.8 vs 778.3 g/bird, P<0.05) and logically broilers from VeO group were heavier than that of CTL one (552.79 vs 530.45 g/bird, P<0.05). However, the lower feed intake measured from CTL group allowed to reduce the relative weight of digestive organs compared to VeO group (11.90 vs 13.80 %LW, P<0.05) optimising carcass yield (78.67 vs 76.19 %LW, P<0.05). Mortality rate from VeO group tended to decrease compared to CTL one (0.52 vs 2.71%, NS). This result indicated this specific neurosensory CE is an efficient solution to support feed intake and growth performance (P<0.05) of broiler under heat stress conditions.

Key Words: neurosensory, citrus extract, heat stress, broiler performance, blood chemistry

352P Peanut skins as a natural antimicrobial feed additive to reduce the transmission of *Salmonella* in poultry meat produced for human consumption. Adam Redhead*¹, Thien Vu², Fernanda Santos³, Ramon D. Malheiros⁴, Ondulla Toomer²; ¹*Animal Biosciences & Biotechnology Laboratory, USDA - ARS, Beltsville, Maryland, United States*, ²*Food Science & Market Quality Handling Research Unit, USDA - ARS, Raleigh, North Carolina, United States*, ³*Food, Bioprocessing and Nutrition Sciences Department, North Carolina State University, Raleigh, North Carolina, United States*, ⁴*Prestage Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, United States*.

Salmonella is the leading cause of bacterial food-borne zoonoses in humans and is estimated to account for 35% of hospitalizations and 28% of foodborne illnesses in the U.S. Therefore, the development of effective and applied strategies to control and mitigate bacterial infections in the poultry meat production industry is essential. The peanut industry generates approximately 40 to 70 million pounds of peanut skin waste annually in the U.S. which is predominately discarded. However, peanut skins and extracts contain polyphenolic compounds which have been identified to have antimicrobial properties. The main objective of this study was to determine the antibacterial effect of peanut skins and/or extracts in the diets of broilers on *Salmonella enteritidis* (SE) transmission or proliferation in poultry housing or organs. One hundred sixty male hatchlings (Ross 308) were obtained and randomly assigned to one of the following experimental treatments, (1) PS: peanut skin diet without oral SE oral inoculation (2) PSSE: peanut skin diet and SE oral inoculation (3) CON: control diet without SE oral inoculation (4) CONSE: control diet with SE oral inoculation. Feed intake and body weights were determined at week 0 and 6. On days 10 and 24 post hatch, 3 birds/pen (24 total) from each treatment group were euthanized and the liver, spleen, small intestine, and ceca were collected. The weights of the liver, spleen and ceca were recorded. Organ invasion was determined by counting SE colonies. Each pen served as an experimental unit and was analyzed using a t-test. Performance data was analyzed in a completely randomized design using a general linear mixed model to evaluate differences. There were no significant differences (P > 0.05) in weekly average pen body weight, total feed consumption, bird weight gain and feed conversion ratio between the treatment groups. There were no significant differences in SE CFU/g for fecal, litter or feed between treatment groups CONSE and PSSE. However, for both fecal and litter, the PSSE treatment group tended (P ≤ 0.1) to have a lower colony forming units (CFU)/g *Salmonella* compared to the CONSE treatment group. The results indicate that peanut skins may have potential application as an antimicrobial feed additive to reduce the transmission or proliferation of SE in poultry environments or flocks.

Key Words: *Salmonella*, Peanut Skins, Broiler Chickens, Feed Ingredients, Alternative Feed Ingredients

353P Gompertz-Laird and Von Bertalanffy models to describe the growth curve of Creole hens of Mexico. Diego Zárate-Contreras*¹, Juan M. Cuca-García¹, Arturo Pro-Martínez¹, Gustavo Ramirez-Valverde¹, Omar Hernández-Mendo¹, Jaime Gallegos-Sánchez¹, Rosalía Ordaz-Contreras¹, Belén López-Pérez¹, Fernando González-Cerón²; ¹*Livestock Program, College of Postgraduates Campus Montecillo, Texcoco, State of Mexico, Mexico*, ²*Department of Animal Science, Chapingo Autonomous University, Texcoco, State of Mexico, Mexico*.

The growth is an increase in the cell number of an organism, from embryo stage to adulthood, this process can be explained mathematically by nonlinear models (NLM). The objective of this study was compare two data recording intervals (RID): Gompertz-Laird (NLGM) and Von Bertalanffy (NLVM) applied to a growth curve of Creole hens of Mexico, when two data recording intervals (RID) are used. A total of 116 female birds were used. They ranged from 0 (hatching) to 133 days of age. Animals were fed a diet containing 21.5% CP and 2,900 kcal ME/kg. Water and feed were provided *ad libitum* during the study period. From 0 to 63 days of age, individual live weight (LW) (g) was recorded every third day and also every week. Later, LW was recorded weekly until 133 days of age. Two LW databases with different recording interval were established to estimate the parameters of NLGM and NLVM. In order to achieve this, LW data from some days were not taken into account, the data was considered missing. The recording intervals were: RID₁, every third day (0-63 days of age) and every fourteen days (63-133 days of age) and RID₂, every seven days (0-133 days of age). The estimated parameters of the NLGM include: hatching weight (W_0 , g), asymptotic weight (W_A , g), age at maximum growth (t_i , d), initial growth rate (L , g/d), rate of decay (K , g/d) and in the NLVM: asymptotic weight (W_A , g), age at maximum growth (t_i , d), maximum relative growth (K g/d), live weight at maximum age growth (W_1 , g), integration constant (B). The criteria for selecting the best NLM in each RID was Arkaike information criterion (AIC) and Bayesian information criterion (BIC). The number of observations in each database was RID₁, $n = 3132$ and RID₂ $n = 2320$. Data was analyzed using the NLIN procedure of SAS (Marquardt algorithm). Estimated parameter values in NLGM were: W_0 , RID₁: 33.1313, RID₂: 32.2064; W_A , RID₁: 1843.19, RID₂: 1832.79; t_i , RID₁: 58.3584, RID₂: 58.0980; L , RID₁: 0.095788, RID₂: 0.097152; K , RID₁: 0.023835, RID₂: 0.024039, for the NLVM were: W_A , RID₁: 2136.44, RID₂: 2049.99; t_i , RID₁: 55.3949, RID₂: 53.2330; K , RID₁: 0.016077, RID₂: 0.017133; W_1 , RID₁: 633.021, RID₂: 607.404; B , RID₁: 0.81218, RID₂: 0.82981. Selection criteria values in RID₁ were: AIC, NLGM: 38129.09 vs NLVM: 38149.03 and BIC, NLGM: 38153.29 vs NLVM: 38173.23. In RID₂, AIC was NLGM: 29615.12 vs NLVM: 29627.60 and BIC, NLGM: 29638.12 vs NLVM: 29650.60. In conclusion, the Gompertz-Laird is the best model that describes the growth curve of Creole hens of Mexico with two data recording intervals.

Key Words: Growth curve, Nonlinear models, Gompertz-Laird, Von Bertalanffy, Creole hens

354P Estimation of parameters of the Gompertz model to describe the growth of Creole chickens of Mexico of two generations in random mating. Juan C. Perrusquia Delgado*², Diego Zárate-Contreras¹, Arturo Pro-Martínez¹, Juan M. Cuca-García¹, Eutimio Olivera-Santiago², Giselle G. Maldonado-Martínez¹, Ana M. Rodríguez-Velázquez², Gerardo Aguilar-Villarreal¹, Fernando González-Cerón²; ¹Livestock Program, College of Postgraduates Campus

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The random mating dosen't have a productive orientation, and dosen't present selection, it induces genetic variability in the progeny. The objective of this study was estimated parameters and selection criteria of the nonlinear Gompertz growth model (NLGM) of two generations of Creole chickens (GCC), in random mating. The population of GCC₂ was established with males and females of GCC₁, and this last one with birds coming for diferent place. One hundred forty males chicks of each generation was used, were reared from 0 (hatching) to 63 d of age. Animals were fed a diet containing 21.5% CP and 2,900 kcal ME/kg. Water and feed were provided *ad libitum* during the study period. The conditions of lodging was similar in both generations. Individual live weight (LW) (g) was recorded every seven days. The estimated parameters of NLGM were: hatching weight (W_0 , g), asymptotic weight (W_A , g), age of maximum growth (t_i , d), initial growth rate (L , g/d) and rate of decay (K , g/d). The Akaike information criterion (AIC) and the Bayesian information criterion (BIC) were estimated for each GCC. The number of observation for each GCC was 1400. Data were analyzed using the NLIN procedure of SAS (Marquardt algorithm). Estimated parameter values were as follows: W_0 , GCC₁: 31.93, GCC₂: 27.56; W_A , GCC₁: 2539.97, GCC₂: 2142.42; t_i , GCC₁: 62.58, GCC₂: 55.34; L , GCC₁: 0.10, GCC₂: 0.11 and K , GCC₁: 0.023, GCC₂: 0.026. The values of AIC and BIC were GCC₁: 16629.55, GCC₂: 16144.23 and GCC₁: 16650.53, GCC₂: 16165.21, respectively. In conclusion, the populations in random mating tend a modify the magnitude of the parameter and of the selection criteria AIC and BIC in the Gompertz model according increase the generations.

Key Words: Growth curve, Nonlinear Gompertz model, Creole chickens, Random mating, Gallus gallus domesticus

355P The effects of rearing system design on bone mineral density and skeletal development in laying hens.

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As the US laying hen industry transitions from conventional cage to multi-tier aviary housing, it is important to rear pullets in systems that prepare them to negotiate these more complex environments. We investigated the effect of rearing system on bone mineral density (BMD) and skeletal development in Dekalb White hens at 8, 18 and 30 weeks of age (woa). We hypothesized that pullets reared in multi-tier and single-tier aviary environments would have higher

BMD of the humerus, keel and tibia than those reared in floor pens due to increased opportunities for making vertical transitions and thus, increased bone loading. We also expected BMD and bone length to increase with age. Pullets (n=804) were raised in groups of 56 in 3.05 x 3.05 m² either in pens furnished with five 10cm high perches, single tier aviaries with elevated perches, or double tier aviaries with elevated perches (5 pens/treatment). At 16 woa, pullet housing was replaced with four-tier aviary systems. At 8, 16, and 30 woa, the keel, both tibiae, and both humeri were collected from 60 pullets/hens per treatment group. Only hens deemed to have intact bones by palpation were used in the study. Bone measurements and BMD of the full tibia, center of the tibial diaphysis, full humerus, center of the humerus, and full keel bone, and the lengths of these bones were measured using Dual-Xray Absorptiometry by a single researcher. A test of intra-rater reliability yielded an interclass correlation value of >0.90. Data were analyzed using linear mixed effects models in R. We found a treatment*age interaction for all BMD measurements, and keel bone length. The length and BMD of the tibia, humerus and keel increased with age. At 16 woa, BMD of the full tibia (p=0.003), tibial diaphysis (p=0.012), full humerus (p=0.004), and non-fractured keel bones (p=0.013) were lower in floor-reared pullets than those reared in single or multi-tier aviaries. At 30 woa, single-tier aviary reared hens had shorter keel bones than those reared in the multi-tiered aviary and floor pens (p = 0.008). The lengths of the tibia and humerus increased from 8 to 16 woa, and did not differ between treatments at any age. In conclusion, access to tiered structures and thus, the opportunity to utilize vertical space, promoted bone development between 8 to 16 weeks of age.

Key Words: laying hen, rearing, keel, housing, bone mineral density

356P Performance of Japanese Quail subjected of environmental high temperature and different nutrition plans. Romilton Barros Júnior², João P. Souza Silva¹, Tamires M. Silva Felix¹, Jose A. de Lira Barbosa¹, Fernando Guilherme Perazzo Costa¹, José H. Vilar Da Silva¹; ¹*Animal Science, Universidade Federal da Paraíba, Solânea, Paraíba, Brazil*, ²*UFPPB, MACEIÓ, AL, Brazil*.

One of the great challenges for quail producers in Brazil and in the tropical world is the high environmental temperatures inside the shed. Understanding how the interaction between diet and heat affects growth and egg production can help reduce economic losses for breeders. In addition, there are several feed plan options for quail in the literature, while some authors recommend two diets, others recommend a single diet during the growth phase. Therefore, choosing the best feed plan is still a controversial decision for quail breeders. The experiment aimed to evaluate the grower of Japanese quail fed different nutritional plans (NPs) at two environmental temperatures. The NPs evaluated were: NP₁ = 2 diets (1 to 21 and 22 to 39 day); NP₂ = 2 diets (1 to 14 and 15 to 39 day); and NP₃ = single diet (1 to 39 day).

The NP₁ and NP₃ diets were recommended by Brazilian Tables for Japanese and European Quail (Silva & Costa, 2009) and NP₂ followed the suggestions of the Brazilian Poultry and Pork Tables (Rostagno et al., 2017). A total of 288 birds were housed in an environment of 25°C, considered to be of thermal comfort, and another 288 birds were housed at a continuous temperature of 35°C, considered temperature of heat stress. The experimental design was completely randomized in a 3 × 2 factorial scheme resulting in 6 treatments with 12 replicates of 8 birds. Feed intake, weight gain and feed conversion from 1 to 39 days of age were evaluated. The data obtained were subjected to analysis of variance and the effects of treatments were submitted to the Tukey test at 5%. The weight gain of quail was higher (p<0.05) when fed with NP₃ compared with NP₂ (154.49^a g vs. 150.21^b g) and better feed conversion when fed with NP₁ (2.98^a g/g) in comparison with NP₂ (3.14^b g/g) and NP₃ (3.04^b g/g). The age of switching from the initial to growing diet between the feed plans may have been determinant for the lower performance of the NP₂ quail (switching at 14 days). Birds submitted to high temperature (35°C) decreased (p<0.05) feed consumption (412.03^b vs. 523.85^a g) and weight gain (144.82^b vs. 160.60^a g) compared to those kept in thermal comfort, respectively. Japanese quail fed with NP₁ and NP₃ show better performance in thermal comfort (25°C) and in high temperature (35°C) compared to NP₂.

Key Words: nutrition, quail meat, weight gain, feed conversion, temperature

357P Characterization of growth patterns and carcass characteristics of male and female broilers from four commercial strains fed low or high density diets. Clay J. Maynard^{*1}, Craig W. Maynard¹, Ashunti Jackson², Michael T. Kidd¹, Samuel J. Rochell¹, Casey M. Owens¹; ¹*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*, ²*Cobb Vantress, Inc., Siloam Springs, Arkansas, United States*.

Over the last few decades, the poultry industry has seen the emergence of various market segments that are beneficial for rearing various flock sizes. Two concurrent experiments consisting of 1,200 broilers were conducted to evaluate the effects of sex and diet on the performance of four commercially available broiler strains, including standard yielding (SY) and high yielding (HY) strains. Within each experiment, a small bird and big bird debone market were targeted to give variable carcass size. Therefore, a three-way ANOVA was utilized for both experiments to assess the effects of sex, diet, and carcass size in both experiments. Two common polyphasic diets were assessed using varying levels of digestible Lys. The low-density diet (LD) consisted of 1.20, 1.10, 1.00, and 0.96% digestible Lys and the high-density diet (HD) consisted of 1.32, 1.21, 1.10, and 1.06%. Weekly BW, BW gain, feed intake (FI), FCR, and processing yields were assessed during both experiments. Broilers fed the HD diets responded better than those fed the LD diets, regardless of sex, with increased BW and

decreased FCR ($P < 0.05$). Additionally, males converted a higher BW with a lower FCR ($P < 0.05$) than their female cohorts. Male HY strains provided the highest carcass yields ($P < 0.05$) compared to SY strains, with no differences observed in females ($P > 0.05$). High density diets also produced increases in carcass, breast, and tender yield ($P < 0.05$) for males but was not present for females ($P < 0.05$). Overall, the impact of strain had the highest effect on performance traits and carcass yields. Therefore, the use of specific strains for various market segments is beneficial for integrators to maximize return.

Key Words: broiler, amino acid density, carcass, strain, yield

358P No presentation materials submitted.

359P Epiphyseal and diaphyseal bone mineral density comparison of turkey Femurs. Dalton Piotter* ^{UG 2}, Gabriella Furo³, Darrin M. Karcher⁴, Jeanine A. Brannon¹, Sally L. Noll¹; ¹*Animal Science, University of Minnesota, Saint Paul, Minnesota, United States*, ²*University of Minnesota, Saint Paul, Minnesota, United States*, ³*Animal Science, University of Minnesota, Debrecen, Hungary*, ⁴*Purdue University, West Lafayette, Indiana, United States*.

Bone mineralization is considered highly characteristic of skeletal structural integrity to support increasing BW and muscle growth. In turkeys, the femur is susceptible to fracture increasing interest in characterizing femur sections. The objective of this experiment was to compare the bone mineralization of the femurs between four different equal length sections of the bone. The epiphyseal region (25% of end-region) was hypothesized to have a lower mineral density than the diaphyseal region (50% mid-region). Turkeys from seven commercial hen farms were euthanized and both femurs dissected (2-10 birds per farm) for a total of 37 hens near market age (average age of 113 days). The tissue was removed, and the femurs were cut into 4 sections designated distal and proximal epiphyseal and two diaphysis. The bones were extracted with ethyl alcohol followed by anhydrous ether and ashed at 600°C and percent ash calculated. Subjected to statistical analysis were bone ash percentage, differences in farms, asymmetry between left and right femurs, and relationship of bone ash percentage with BW. An analysis of variance was run for each left and right femur (Statistix 10) with bone section or farm as the main factor. Tukey HSD all-pairwise comparisons test and contrasts were used to detect mean differences for bone ash. Paired T-test and correlation (Pearson) was used to assess asymmetry of left and right bone ash content. Bone ash was regressed on BW. Bone sections differed in percentage ash ($P < .00001$). The differences in bone ash percent among farms was not significant. Tukey's HSD All-Pairwise Comparisons Test found two homogeneous groups between the two epiphyseal (distal=43.98; proximal=43.98) and two diaphyseal (distal=61.12; proximal=59.87) sections for average left and

right bone ash percentage. The ANOVA contrasts T-statistic for bone ash comparing epiphyseal and diaphyseal regions indicated statistical significance between the two bone regions ($P < .0001$). Paired T-test indicated similar ash content between the left and right femur. Ash content was highly correlated ($r = .96$) meaning that there was no significant asymmetry between left and right femurs. Regression of ash on BW was not significant, indicating that at an average sample age of 113 days, bone ash content had plateaued. The hypothesis was supported that the epiphyseal region sections had a statistically lower mineral density than the diaphyseal region sections. Under commercial production systems, similar bone ash content was observed across farms and asymmetry in bone ash content of the left and right femur was not apparent. Funding provided by USDA NIFA no. 2016-67015-24457.

Key Words: Turkey, Femur, Bone mineralization, Epiphyseal, Diaphyseal

360P Initial evaluation of Pulsed Alternating Wavelength System (PAWS) on growth and physiological markers of stress in grow-out Pekin ducks. Sara Tonissen* ^{UG 1}, Jason Suntych², Daren Suntych², Marcus Reinhardt², Darrin M. Karcher¹, Gregory S. Fraley¹; ¹*Animal Sciences, Purdue University, West Lafayette, Indiana, United States*, ²*Xiant Technologies, Inc., Greeley, Colorado, United States*.

Lighting can have profound effects on the health, production, and welfare of all poultry species, and the duck is no exception. Like all poultry species, ducks are seasonal breeders grown in curtain sided barns, thus require artificial light in order to maintain long daylengths to maximize growth and fertility. However, there are no standards for lighting in commercial duck barns. Very few studies have evaluated the growth and welfare of ducks housed under different lighting systems. However, recent studies from several labs have reported that monochromatic light, particularly blue light, may not be appropriate for ducks, or waterfowl in general. A novel LED technology has recently been introduced referred to as Pulsed Alternating Wavelength System (PAWS) that provides a new approach to delivering multiple wavelengths of light to animals. The goal of this study is to determine the effects of PAWS on growth, FCR, and welfare of grow-out Pekin ducks. Five unique spectral recipes were evaluated with 40 ducks per light treatment: industry control (fluorescent light 18L:6D) PAWS 1 (24L), PAWS 2 (24L), PAWS 3 (18L:6D) and PAWS 4 (18L:6D). Body weight and FCR were calculated weekly on all ducks. At processing age, (day 30), we evaluated ($N = 5$ per treatment) heterophil:lymphocyte ratios, spleen and bursal weight, and collected brains for biogenic amine analyses within the caudal raphe/ventral tegmental area, dorsal raphe, diencephalon, and forebrain. All analyses were done at the level of the duck and evaluated using 2-way repeated measures ANOVA (BW and FCR), and 2-way ANOVA for physiological data. No differences were observed in BW, FCR, or relative spleen

or bursal weights among the treatment groups. There was a decrease in HLR in PAWS treated ducks compared to controls that approached significance ($p = 0.1$). No differences were observed in any PAWS treatment for biogenic amines, thus final data were combined for description. No differences in norepinephrine or epinephrine were observed between PAWS and controls. There was a significant increase ($p < 0.05$) in dopamine levels in the VTA and forebrain in PAWS compared to controls. A non-significant ($p = 0.061$) increase in serotonin levels were observed in the dorsal raphe. Thus, there were no physical or physiological signs of acute or chronic stress; the most important outcome of this study is that the novel PAWS technology has no negative impact on production nor does PAWS elicit any physiological signs of stress. This was a small initial trial, but these data combined with the possibility that PAWS may impact brain biogenic amines warrants future larger studies.

Key Words: serotonin, dopamine, norepinephrine, mass spec

361P Dietary ginger root extract modulates fecal concentrations of *Bifidobacteria* and *Lactobacillus* in laying hens. George Dosu*^{GS}, Shengmin Sang, Temitayo Obanla, Tiffany Crenshaw, Yewande Fasina; *North Carolina A&T State University, Greensboro, North Carolina, United States.*

It has been established that ginger (*Zingiber officinale*) contain bioactive compounds (such as gingerols and shagoals) that impart antimicrobial properties and may therefore replace antibiotics in laying hen diets. A 21-day experiment was conducted with 56-weeks old laying hens (48; White Leghorn) to evaluate the effect of ginger root

extract (**GRE**) supplementation on fecal concentrations of total bacteria, *Escherichia coli* (indicator of presence of pathogenic bacteria), and immune-enhancing bacteria species (*Bifidobacterium* spp. and *Lactobacillus* spp.). Hens were weighed and randomly allocated to four dietary treatments. Treatment 1 (**CON**) consisted of hens given conventional layer corn-soybean meal (**SBM**)-based diet without GRE. A similar diet supplemented with GRE at 0.1%, 0.3%, and 0.9% was given to hens in Treatments 2 (**GRE1**), 3 (**GRE2**), and 4 (**GRE3**), respectively. Each treatment consisted of 4 replicate pens, with each pen housing 3 laying hens. On d 21 of experiment, fecal samples were collected, serially diluted, and plated onto Brain Heart Infusion (**BHI**), McConkey, deMan–Rogosa–Sharpe (**MRS**), and modified Bifidobacterium Iodoacetate Medium-25 (**mBIM-25**) agar plates for the enumeration of total bacteria count, *Escherichia coli*, *Lactobacillus* spp., and *Bifidobacterium* spp., respectively. Plates were incubated for 24 to 72 h, and colonies were counted thereafter. Results showed that hens given diets supplemented with GRE at 0.1%, 0.3%, and 0.9% level had lower fecal population of total bacteria and *E. coli* ($P < 0.05$) compared to the CON (control) treatment. Hens in CON treatment (6.57 Log₁₀ CFU / g of fecal content) had fecal *Bifidobacterium* spp. concentration that was similar ($P > 0.05$) to those in GRE1, GRE2, and GRE3 treatments (6.15 to 7.28 Log₁₀ CFU/g). Fecal concentration of *Lactobacillus* was higher ($P < 0.05$) in GRE1 (6.60 Log₁₀ CFU / g) and GRE2 (6.58 Log₁₀ CFU / g) compared to CON hens (5.97 Log₁₀ CFU / g). It was concluded that dietary GRE levels up to

Key Words: Laying hens, Ginger root extract, *Escherichia coli*, *Bifidobacterium* spp., *Lactobacillus* spp.

Metabolism and Nutrition: Amino Acids

362P Effects of dietary L-Citrulline supplementation on the nitric oxide synthesis and mitochondrial bioenergetics in the breast muscle of heat stressed broilers. Victoria A. Uyanga*, Jingpeng Zhao, Xiaojuan Wang, Hongchao Jiao, Hai Lin; *Animal Nutrition and Feed Science, Shandong Agricultural University, Tai'an, China.*

L-Citrulline, a non-protein amino acid, serves as a potent substrate for arginine recycling, and also functions in enhancing muscle protein synthesis. The objective of this study was to determine the effects of heat stress on the nitric oxide regeneration and muscle bioenergetics of broilers and to assess whether dietary L-Citrulline can mediate these effects. Arbor acre broilers (288 males, 1d) were fed either basal diet (control) or basal diet supplemented with 1% L-Citrulline (L-Cit). At 28 d, broilers were subjected to two environmental temperatures, 35 °C for 8 h/d (HS) or 24 °C (TNZ) for 14 days in a 2 x 2 factorial design. Body temperature was regularly monitored, while the blood and breast muscle samples were collected after 14 d HS. Core body (T_b) and rectal temperatures rose immediately during HS exposure, but under TNZ condition, L-Cit suppressed T_b by ~0.5°C decline, initiating sustained hypothermia in broilers. Plasma metabolites for glucose, blood urea nitrogen, and lactate dehydrogenase were affected ($P > 0.05$) by HS and L-Cit supplementation. Bodyweight gain, average daily feed intake (ADFI) and feed conversion ratio (FCR) were reduced by HS exposure ($P > 0.05$), however, L-Cit supplementation alleviated HS-depression of ADFI and FCR. Abdominal fat weight was decreased by HS, but HS and L-Cit increased ($P > 0.05$) the kidney and heart weight of broilers. Nitric oxide (NO) concentration was increased during HS condition ($P > 0.05$), whereas, NO synthase (NOS) activities were diminished by HS exposure, except for endothelial NOS ($P > 0.05$). Dietary L-Cit increased the endothelial and inducible NOS isoforms ($P > 0.05$) compared to the control fed group. Also, the mRNA expression for mitochondrial biogenic genes including PGC-1 α , ANT, and COX III were downregulated ($P < 0.05$) in the breast muscle during HS, whereas, L-Cit significantly induced TFAM expressions compared to the control fed group. The mRNA expression for ATP5 β was differentially affected ($P < 0.05$) by L-Cit supplementation at HS and TNZ conditions. Under TNZ condition, L-Cit downregulated ATP5 β expression, whereas, under HS condition, L-Cit upregulated ATP5 β expression in comparison to the control fed broilers. Therefore, this study demonstrates the modulatory effects of L-Citrulline on body temperature, nitric oxide synthesis, and mitochondrial bioenergetics in the breast muscle of broilers. The study also reveals the possible association between L-Cit-induced hypothermia and muscle ATP generation during thermoneutral condition.

Key Words: Citrulline, Body temperature, Mitochondrial bioenergetics, Nitric oxide, Heat stress

363P Determining amino acid digestibility of soybean meal from different South Carolina soybean varieties when fed to broilers. Kara M. Dunmire*^{GS 1}, Michaela B. Braun¹, Caitlin E. Evans¹, Benjamin D. Fallen², Charles R. Stark¹, Chad B. Paulk¹; ¹*Grain Science and Industry, Kansas State University, Manhattan, Kansas, United States*, ²*Plant and Environmental Sciences, Clemson University, Clemson, South Carolina, United States.*

At hatch, 240 Ross 308 male broilers were placed in battery cages for a 15-d study to evaluate amino acid digestibility of soybean meal from specialty variety soybeans grown in South Carolina. There were 10 replicates per treatment and 6 broilers per cage. Broilers were given a common corn soybean meal-based diet from d 0 to 9. On d 9, broilers were weighed, and cages were allotted to 1 of 4 dietary treatments within location block. Soybean meal (SBM) sources consisted of a conventionally processed control SBM (45.4% CP; CON), and soybeans sourced from the same region, processed using solvent extraction at Texas A&M University (49.8% CP; PCON). Two additional soybean varieties were solvent extracted at Texas A&M and resulting SBM (53.1% CP; 53SBM and 57.4% CP; 57SBM) used in this experiment. Assay diets were dextrose and SBM-based and consisted of 1 of 4 soybean meal sources formulated to supply 20% dietary CP with titanium dioxide as an indigestible marker. On d 15, broilers were euthanized by CO₂ inhalation and ileal samples were collected to determine apparent ileal digestibility (AID) of amino acids (AA). Data were analyzed using the GLIMMIX procedure in SAS 9.4, with pen as the experimental unit, pen location as the blocking factor and adjusted using Tukey-Kramer multiple comparisons. Broilers fed CON and 57SBM had increased ($P < 0.003$) AID of total AA (77.3 and 81.5% vs. 71.6 and 69.0%), arginine (85.2 and 88.5% vs. 80.5 and 79.6%), histidine (80.2 and 83.1% vs. 74.1 and 72.2%), lysine (79.7 and 82.2% vs. 71.4 and 69.2%), threonine (77.4 and 80.0% vs. 69.8 and 66.2%), and tryptophan (75.7 and 82.5 vs. 73.8 and 72.6%) compared to those fed PCON and 53SBM. The AID of isoleucine and phenylalanine increased ($P < 0.001$) in broilers fed 57SBM (81.6 and 83.2%) compared to CON (76.9 and 77.7%) and 53SBM (73.5 and 72.5%), where PCON (72.8 and 74.7%) was intermediate to CON and 53SBM. Broilers fed 57SBM had increased ($P < 0.001$) AID of leucine (82.1%) compared to all other sources. The AID of methionine increased ($P = 0.007$) in broilers fed CON (80.2%) and 57SBM (81.3%) compared to 53SBM (70.9%), where there was no evidence for differences between those fed PCON (73.8%) and all other sources. Broilers fed CON (74.2%) and 57SBM (79.1%) had increased ($P < 0.001$) digestibility of valine compared to PCON (69.7%) and 53SBM (66.3%) with no evidence for difference between those fed CON and PCON. In conclusion, broilers fed commercially processed soybean meal had improved AA digestibility compared to those fed experimentally processed soybeans from a similar region.

The high CP (57% CP) SBM variety had increased AA digestibility compared to the PCON and 53SBM.

Key Words: broiler performance, amino acid digestibility, soybean meal

364P Determination of standardized ileal digestibility of tryptophan in granular sources for 21-day-old broiler chickens. June Hyeok Yoon*¹, Hyeon-Jin Kim², Hyelim Lee², Changsu Kong³; ¹*Animal Science and Technology, Konkuk University, Seoul, Korea (the Republic of)*, ²*Animal Nutrition Solution Team, CJ BIO, Seoul, Korea (the Republic of)*, ³*Animal Science, Kyungpook National University, Sangju, Gyeongsangbuk-do, Korea (the Republic of)*.

The objective of this study was to determine standardized ileal digestibility (SID) of Trp in granular sources by using direct and regression methods and to compare the methods for determining the digestibility of Trp. Three hundred seventy-two 19-day-old Ross 308 male broiler chickens (initial body weight = 506 ± 82.7 g) were assigned to eight experimental diets with nine birds per cage for the direct method or seven birds per cage for the regression method in a randomized complete block design with body weight as a blocking factor. Eight experimental diets consisted of a nitrogen-free diet and two semi-purified diets for the direct method and five corn-soybean meal (SBM)-based diets for the regression method. Two semi-purified diets were formulated to contain calcium (Ca) Trp granular complex (Ca-Trp; 60% purity, 69.5% CP, and 1.88% Ca) or granular Trp (60% purity, and 75% CP) as a sole source of amino acid. For the regression method, a basal diet without supplemented granular Trp were formulated, and four experimental diets were contained two levels (3 and 6%) of supplemented granular Trp from sources at the expense of corn and SBM. Chromic oxide was added to experimental diets as an indigestible index. No differences were found between Ca-Trp and granular Trp for SID of Trp in the direct method (99.33 and 99.61%). There were linear ($P < 0.05$) and quadratic ($P < 0.05$) increases in SID of Trp as both Trp sources increased, respectively. No difference was observed for the digestibility derived from regression method between Ca-Trp and granular Trp (101.49 and 101.05%). Compared direct method to regression method, obtained digestibility from Ca-Trp were differ ($P < 0.05$), while the digestibility from granular Trp did not differ. In conclusion, the digestibility from the direct method or the regression method did not differ between Trp sources. The digestibility of Ca-Trp from the direct method were different from those obtained by the regression method, while the digestibility of granular Trp were not different.

Key Words: tryptophan, direct method, regression method, standardized ileal digestibility, broilers

365P Standardized ileal digestible methionine requirements of male broilers from 22 to 29 days. Su Hyun An*¹, Hwang-Ku Kang³, Changsu Kong^{2, 1}; ¹*Animal Science and Biotechnology, Kyungpook National University, Sangju, Gyeongsangbuk-do, Korea (the Republic of)*, ²*Animal Nutrition Solution Team, CJ BIO, Seoul, Korea (the Republic of)*, ³*Institute of Poultry Science, National Institute of Animal Science, Pyeongchang, Gangwon-do, Korea (the Republic of)*.

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The objective of the present study was to estimate the standardized ileal digestible (SID) methionine (Met) requirement for male broilers (Ross 308) at the age of 22-29 days. A total of 192 birds (745 ± 50.5 g) were allocated to six dietary treatments in a randomized complete block design with eight replicate cages per treatment. The six experimental diets were formulated based on corn, soybean meal, and synthetic amino acids to contain dietary SID Met equally increased from 0.40 to 0.65% with a consistent concentration of all amino acids except for Met. Data for weight gain (g/d), feed intake (g), gain to feed ratio (g/kg), and relative feather weight (% of body weight) from 22 to 29 d of age broilers were fitted to linear and quadratic response curves using the GLM procedure of SAS. When SID Met increased in the experimental diet, the weight gain and feed intake increased quadratically ($P < 0.05$). On the other hand, the gain to feed ratio (g/kg) and relative feather weight (% of body weight) did not differ in response to the dietary SID Met concentration. The SID Met requirements for gain and feed intake were 0.454% and 0.456% for the one-slope broken-line, 0.546% and 0.549% for the quadratic-line, 0.519% and 0.522% for 95% of the upper asymptote of the quadratic model, respectively. In conclusion, the SID Met requirements for broilers fed corn-SBM-based diets from 22 to 29 days ranged from 0.454% to 0.546% for weight gain and from 0.456% to 0.549% for feed intake.

Key Words: Amino acid, Requirement, Methionine, Male, Broiler

366P Protective effect of L-Citrulline on the growth performance of cyclic heat stressed broilers via modulation of hypothalamic GH/IGF-1 pathway proteins. Victoria A. Uyanga*^{GS}, Jingpeng Zhao, Xiaojuan Wang, Hongchao Jiao, Hai Lin; *Animal Nutrition and Feed Science, Shandong Agricultural University, Tai'an, China.*

Heat stress is typically associated with adverse effects on the growth rate and production performance of poultry, and an identification of feed additives to ameliorate these detrimental effects is crucial. L-Citrulline is a nutraceutical amino acid that is widely gaining research interest for its role in arginine recycling and muscle protein synthesis. This study was designed to investigate whether dietary supplementation with L-Citrulline could mediate the adverse effects of heat stress on the growth performance of broilers. Arbor acre broilers (288 males, 1d) were raised on two diets; basal diet (control) or basal diet supplemented with 1% L-Citrulline (L-Cit). After 3 wks pre-feeding, the birds were subjected to two environments; thermoneutral at 24°C (TNZ), or heat stress at 35°C (HS) for another 3 wks, in a 2 x 2 factorial design. Weekly data was obtained for

body weight and feed intake, and the blood and hypothalamic tissues were collected at the 6th week of experiment. Results showed that HS significantly lowered ($P > 0.05$) the feed intake for both control and L-Cit fed groups except at the 4th wk of age. L-Cit supplementation reduced ($P > 0.05$) the feed intake at the 2nd wk prior to HS exposure, but increased the feed intake at the 4th week of age compared to control fed group. HS exposure decreased the body weight of broilers from the 4th to 6th week of age, whereas L-Cit supplementation increased the body weight gain, especially under TNZ condition. HS and L-Cit treatment influenced the hypothalamic expression of Pro-opiomelanocortin (POMC) neuropeptide ($P > 0.05$), but did not affect Neuropeptide-Y and Ghrelin expression. Compared to TNZ condition, HS significantly lowered POMC expression but this effect was alleviated with L-Cit supplementation. Analysis of the growth hormone/ insulin-like growth factor-1 (GH/IGF-1) pathway showed that HS downregulated ($P > 0.05$) the hypothalamic mRNA expression of Growth Hormone Receptor (GHR), Growth Hormone Binding Protein (GHBP), Insulin-like growth factor-1 (IGF-1), and Insulin-like Growth Factor Binding Protein (IGFBP), but upregulated IGF-1 Receptor expression. In contrast, L-Cit supplementation significantly ($P > 0.05$) upregulated GHBP, and IGFBP expression, alleviating HS-induced effects. Therefore, this study demonstrates the protective ability of L-Citrulline supplementation on the growth performance and body size of HS broilers, and that this effect was mediated at the transcriptional level via modulation of hypothalamic GHBP and IGFBP in the somatotropic axis of broilers.

Key Words: L-Citrulline, Amino acids, Somatotropic axis, Hypothalamus, Heat stress

367P Sulphur amino acids sources affect the gene expression of glucose transporters in the jejunum of broilers chicken. Tamires M. Silva Felix^{*UG1}, Claudiana S. Souza¹, Jose A. Barbosa¹, Fernando Guilherme Perazzo Costa¹, Patricia E. Givisiez¹, Danila B. Campos¹, Samuel Aggrey², José H. Vilar Da Silva¹; ¹*Animal Science, Universidade Federal da Paraíba, Solânea, Paraíba, Brazil,* ²*Department of Poultry Science, University of Georgia, Athens, Georgia, United States.*

Methionine is an essential amino acid for the renewal of the

intestinal epithelium and synthesis of glutathione, a major cellular antioxidant. The availability of sulfur amino acids influences the absorption of other nutrients, affecting the growth of birds, however few studies have reported the relationship between methionine sources supplementation and the transporters of nutrients in intestinal cells. The objective was to study the mRNA expression of glucose transporters in the jejunum of broiler chickens whose diets were supplemented with different sources of dietary methionine. A total of 450 male Cobb700 chickens at 8 days of age with an average BW of 180g were used in 2x3 factorial experiment. There were two sources of methionine were DL-Methionine (DL-Met) and DL-2- hydroxy-4-methylthio-butanoic acid (DL-HMTBA). The three dietary levels of total digestible sulfur amino acids (Met+Cys) were: deficient (0.63, 0.58, and 0.52), requirement (0.88, 0.83, and 0.77%) and excess (1.13, 1.08, and 1.02%) for 8-21, 22-33 and 34-49 days, respectively. Each treatment had 5 replicates with 15 birds per replicate. At 49 days of age, 1 bird per replicate, five per treatment, was randomly selected, humanely euthanized and the jejunum taken to total RNA extraction. mRNA expression was done on SGLT1, SGLT5, GLUT2 and GLUT5 genes with beta actin as an internal control. The data was analyzed using the ANOVA test. Across all dietary levels, DL-HMTBA supplementation led to upward expression of SGLT1 and SGLT2 ($P < 0.05$) principally in the chicken group that were fed the methionine-deficient diet compared with the other treatment groups. Regardless of methionine source, GLUT5 was downward expressed ($P < 0.05$) in the chickens fed the methionine-excess diet compared to their counterparts. The methionine source by level interaction showed that SGLT1 was downwardly expressed ($P < 0.05$) in group whose diet were supplemented with excess DL-Met compared to those on DL-Met-deficient or excess diets. However, in the DL-HMTBA group, the SGLT1 gene was upwardly expressed ($P < 0.05$) in the methionine-excess group compared to the deficient group. Methionine source could affect the mRNA expression of some sugar transporters, however such changes in gene expression principally occur in either methionine deficient or methionine-excess diet, but not in the regular diets.

Key Words: Broilers, Glucose transporters, Intestinal epithelium, Sulphur amino acids levels, Sulphur amino acid sources

Metabolism and Nutrition: Enzymes

368P No presentation materials submitted.

369P Dietary Allzyme® Spectrum improved the performance of broiler chickens fed low nutrient diet.

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Allzyme® Spectrum is a naturally fermented product with multiple enzyme activity including carbohydrase and phytase. A study was conducted to investigate the effect of supplementing Allzyme® Spectrum on the growth performance of broiler chickens in a 42d period. Dietary treatments included: 1) corn-soy reference diet having a nutrient level equivalent to commercial level; 2) corn-soy low nutrient diet having 90 kcal/kg less ME and 0.15% less Ca and available P compared to diet 1; 3) diet 2 + 0.02% Allzyme® Spectrum. Eight replicate pens of 22 chickens were randomly assigned to each of the three dietary treatments. Chickens were housed in floor pens in an environmentally controlled room with *ad libitum* access to feed and water. Three phase diets including starter (d 0 – 9), grower (d10 – 21) and finisher (d22 – 42) were provided to chickens during the trial period. Chickens fed the low nutrient diet (diet 2) had lower ($P < 0.01$) weight gain compared to those fed diet 1&3 during each phase and the overall period. The feed intake of chickens fed the low nutrient diet was the same as that of chickens fed diet 1&3 during starter period, but lower ($P < 0.01$) than those fed diet 1&3 during grower phase and overall. Chickens fed the low nutrient diet had higher ($P < 0.01$) feed to gain ratio compared to those fed diet 1&3 during the starter and overall growing period. The results from this study indicated that the supplementation of Allzyme® Spectrum in the diet having low ME, Ca and available P increased weight gain and improved feed conversion ratio of broiler chickens.

Key Words: broiler, performance, enzyme, ME, available P

370P Comparative effects of two phytases on bone mineralization, nutrient digestibility and phytate-P degradation of broilers.

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A battery study was conducted to evaluate the efficacy of two phytases in improving bone mineralization, nutrient digestibility and phytate-P degradation of broilers. At 0-8 day of age, all the birds were fed with a corn-SBM common starter diet containing 0.70% total Ca and 0.23% non-

phytate P (nPP). The experimental diets were provided to birds at 8-18 day of age, including: negative control (NC), positive control (PC), NC + a novel phytase (NPhy) at 187.5, 375, 750, 1500 and 2000 FYT/kg, and NC + a current generation of phytase (CPhy) at 500, 1000 and 2000 FYT/kg. The two phytases were from DSM Nutritional Products Inc. The total Ca and nPP were 0.70% and 0.23% for NC, and 0.90% and 0.43% for PC, respectively. Each diet was fed to 9 pens of 8 birds. On d 18, the digesta from gizzard and distal half of ileum were collected from 8 birds/pen, and the right tibia was taken from 2 birds/pen. Data were analyzed by one-way ANOVA. Additionally, an exponential model was applied for the nonlinear response of each phytase. NPhy responses at the same dosage of CPhy were interpolated from regressions, then data were analyzed as a 2×3 factorial to determine the main effect of phytase source, dosage and their interaction. Means were separated by protected Fisher's LSD test with a value of ≤ 0.05 considered significantly different. One-way ANOVA analysis showed NC had significantly lower tibia ash, tibia P and ileal P digestibility ($P < 0.05$), higher phytate-P degradation in ileum ($P < 0.05$), and no difference for phytate-P content in gizzard ($P > 0.05$) in comparison with PC. All the supplemental levels of NPhy and CPhy significantly increased ($P < 0.05$) tibia ash and P, ileal P digestibility, phytate-P degradation in ileum and decreased ($P < 0.05$) phytate-P content in gizzard when compared with NC. Broilers fed NPhy at 1500 or 2000 FYT/kg and CPhy at 2000 FYT/kg had tibia ash and tibia P comparable with birds fed PC. The two-way ANOVA analysis showed there was a significant interaction between phytase source and dosage for tibia ash ($P = 0.05$) and tibia P ($P = 0.02$), NPhy at 500 and 1000 FYT/kg showing similar tibia ash and tibia P deposition with CPhy at 1000 and 2000 FYT/kg, respectively. There was no interaction between the two factors for ileal P digestibility and phytate-P hydrolysis, while the main effect showed NPhy had a lower phytate-P content in gizzard ($P < 0.001$), a higher P digestibility and phytate-P degradation in ileum ($P < 0.001$) than CPhy. In summary, the novel phytase delivered superior P release compared to the current generation, in terms of bone ash, bone P and ileal P digestibility, which was confirmed by the faster and more complete phytate-P hydrolysis across gastrointestinal segments.

Key Words: phytase, tibia, P digestibility, phytate-P hydrolysis, broilers

371P Sources of corn and soybean meal and carbohydrase enzymes supplementation differently affect growth performance and nutrient digestibility in broiler chickens.

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This study aimed to evaluate the effects of a proprietary admixture of carbohydrase enzymes on different sources (origin) of corn and soybean meal (SBM)-based diets on growth performance and apparent ileal digestibility (AID) of nutrients of broiler chickens. A total of 216 day-old chicks (Cobb 500) were equally distributed to 36 floor pens (9 replicates, 6 birds/pen), and the pens were allocated to 4 dietary treatments in a completely randomized 2×2 factorial arrangement. The treatments included: 2 corn and SBM sources as the main ingredients supplemented without (NC1 and NC2) or with enzymes (NC1+Enz and NC2+Enz). The diets were formulated in three phases (starter, d 0-14; grower, d 15-28; finisher, d 29-42), and all diets contained phytase in the background. Feed and water were provided ad libitum. The birds were housed in floor pens, and a standard commercial broiler rearing environment (temperature, humidity, and light) was managed. The body weight and feed intake were recorded at the beginning of trial and then on days 14, 28, and 42 to determine growth performance and feed conversion ratio (FCR). At 42d of age, ileal samples were collected to determine the AID of nutrients using the marker (TiO₂) method. Data were subjected to multi-factorial analysis of variance and were compared among treatments using the MIXED procedure of SAS with a pen as the experimental unit. There was no effect of treatments on body weight (BW) and average daily gain (ADG) in the starter phase, but supplementation with enzymes increased (P <0.05) both BW and ADG during grower and finisher phases as well as the overall study period. Also, there was an interaction (P <0.05) between ingredient source and enzyme supplementation for BW, ADG, ADFI, and FCR in the finisher phase and ADG and ADFI in the overall study period. Enzyme supplementation significantly reduced (P <0.05) FCR in all stages of the study. There was a significant effect (P <0.05) of both the ingredient sources and enzymes on the AID of nutrients (DM, GE, and CP) as well as ileal AME, ileal AMEn, and N retention at ileum level. Also, a significant interaction between ingredient type and enzymes was found for N retention (P <0.05). In conclusion, there was no notable effect of ingredient sources, but enzyme supplementation positively affected the growth performance parameters of broilers. As the source of corn and SBM influenced the effect of enzymes on nutrient digestibility and there was an interaction between ingredient source and enzyme supplementation, future work should use ingredients from different origins and explore reasons for this in order to further optimize the economic value of carbohydrases in corn/SBM-based diets for broilers.

Key Words: Broiler, carbohydrase, growth performance, nutrient digestibility, nitrogen retention

372P Sources of corn and soybean meal and carbohydrase enzyme supplementation differently affect cecal volatile fatty acid production and microbiota profile in broiler chickens. Rajesh Jha^{*1}, Razib Das¹,

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The aim of this study was to evaluate the effects of carbohydrase enzymes on different sources of corn and soybean meal (SBM)-based diets on gut health parameters of broilers. A total of 216 day-old chicks (Cobb 500) were equally distributed to 36 floor pens (6 birds/pen), and the pens were randomly allocated to 4 dietary treatments in 2×2 factorial arrangement. The treatments included: 2 corn and SBM sources as the main ingredients supplemented without (NC1 and NC2) or with enzymes (NC1+Enz and NC2+Enz). The diets were formulated in three phases (starter, d 0-14; grower, d 15-28; finisher, d 29-42), and all diets contained phytase in the background. The birds were raised under standard husbandry practices and were fed mash feed ad libitum. On d 42, cecal digesta were collected from 9 birds from each treatment (1 bird from each pen) for volatile fatty acids (VFA) and microbiome profiling. The VFA production was different among treatments, but the effect of either ingredient sources or enzyme supplementation was inconsistent, primarily due to high variability among birds within treatments. Enzyme supplementation increased (P <0.01) the production of branched-chain fatty acids in both ingredient sources, while NC2 had a higher level (P >0.05) of total VFA among all treatments. Analysis of 16S rRNA amplicon sequences from cecal digesta did not reveal any difference in alpha or beta diversity of microbiota among treatments (P >0.05). However, enzyme supplementation enriched the mean proportion *Anaerostipes butyraticus* (a butyrate-producing bacteria) compared with no enzyme groups (P <0.01). Relative abundance of *Lactobacillus* was higher in the NC+ENZ group than others. The results suggest that carbohydrase enzyme supplementation affects the cecal VFA production and microbial profile, but the effects may be dependent on the source of corn or SBM in the diet. Thus, ingredients from different origins need to be tested in future studies to validate further enzymes' effects on cecal VFA production and microbiota profile in broiler chickens.

Key Words: Broiler, carbohydrase, gut microbiota, short-chain fatty acids

373P Field evaluation of an exogenous protease in commercial turkey diets. Elizabeth J. Kim^{*}, Franco Mussini, Jordon Gruber, Michael Perry, Janet Remus; *Danisco Animal Nutrition/ IFF, Buford, Georgia, United States.*

The objective of this study were to evaluate the inclusion of an exogenous protease in tom turkey dietson performance and key gut pathogens, such as Avian pathogenic E. coli (APEC), within a vertically integrated system. A paired-house model consisted of a control and test house on each farm was used, for a total 12 houses on 6 farms; all houses fed from the same feed mill. Birds were fed the same commercial diets through all phases and placed under the

integrator's management practices for lighting and temperature. Diets were corn-soybean meal based and were formulated to commercial dietary specifications for each phase, with the test diet formulated with an exogenous protease (Aextra® PRO; 4000 u/kg feed) at recommended nutrient specifications. At 7, 9, 12, and 15 weeks of age, 3 birds were randomly selected from each house and their gastrointestinal tracts (GIT) taken to quantify *E. coli* and characterize the virulence genes to determine potential pathogenicity. All birds were processed at 19 weeks of age. Houses were tracked individually for performance and GIT bacterial quantification and then pooled together for statistical analysis. All data were analyzed via a one-way ANOVA. Means were separated by Tukey HSD using JMP Pro 15 (SAS Institute). The inclusion of exogenous protease had no significant effect on final body weight, and ADG ($P > 0.05$), which was expected due to the similar nutritional composition of the diets. However, there was a significant increase in overall total livability ($P < 0.05$) for market toms fed the test diet with protease of 4%. This increased livability means that for every 100 turkeys placed, an extra 5 birds went to the processing plant at 19 weeks. Additionally, the inclusion of an exogenous protease was found to reduce the virulence of APEC genes and support more commensal *E. coli*, indicating a potential shift in nutrient flow between the turkey and its gut microbial community. Further investigation into the role that protease plays in gut health is needed given the benefit in viability seen in this work.

Key Words: Turkeys, Protease, APEC, *E. coli*, Gut health

374P Performance of commercial laying hens supplemented with functional fiber associated with xylanase. Deibity A. Cordeiro¹, Natiele F. de Oliveira¹, Imar C. Fernandes Filho¹, Lucas B. de Castro¹, Laura A. Duarte¹, Fernanda B. Toledo¹, Thaciane L. Amaral¹, Alexandre B. de Brito², Jose H. Stringhini*¹; ¹*Zootecnia, Universidade Federal de Goias, Goiania, Goias, Brazil*, ²*ABVista, Malborough / Sao Paulo, United Kingdom*.

This study was conducted to evaluate the effects of fermentable fiber associated with a fibrolytic enzyme (xylanase) on the performance of commercial laying hens

from 33 to 49 weeks of age. A performance trial was carried out at the Veterinary and Animal Science Faculty of UFG involving 320 Hy-Line W80 laying hens weighing 1.545 ± 23 g, for a period of 112 days. The hens were allocated to four treatments in a completely randomized design with ten replicates of eight birds each. Two commercial sources of xylanase with the same enzymatic activity (160,000 BXU/g) were used, one of which consisted of a combination of xylanase and fermentable oligosaccharides. Both products were included at the rate of 75 g/t. Phytase at 450 FTU/kg was used considering only the mineral nutritional matrix. The experimental diet was formulated according to recommendations of the Brazilian Tables for Swine and Poultry. Treatments were as follows: Positive Control Diet; Negative control diet (considering the nutritional contribution of the enzyme xylanase); Negative Control Diet + Xylanase; and Negative Control Diet with Xylanase + Fermentable Oligosaccharides. At the end of the entire period, the following performance variables were evaluated: total feed intake (kg), feed intake (g/hen/day), egg production (% and egg/hen/day), mean egg weight (g), mean egg mass (g/hen/day and g/hen/period) and feed conversion (kg/kg and kg/dozen eggs). Feed intake was corrected according to mortality and egg production was recorded daily. The obtained data were subjected to ANOVA and F test at 5% significance, using R-Project free software. There was statistical significance for feed intake and feed conversion in kg/kg and kg/dozen eggs between the diets ($P < 0.05$). The positive control diet provided better results for feed intake and feed conversion (kg/kg and kg/dozen eggs) than the negative control diets and the treatment containing xylanase and xylanase + fermentable oligosaccharides, which showed similar values. Egg production, average egg weight and egg mass, in turn, were not affected by the nutritional treatments. It should be noted that the birds performed similarly under the same environmental conditions and belonged to the same line, which allowed them to maintain their egg production performance. Thus, the use of the enzyme xylanase and xylanase + oligosaccharides is efficient in maintaining egg production indices, average egg weight and egg mass.

Key Words: carbohydrase, oligosaccharides, egg production, stimbiotic

Metabolism and Nutrition: Feed Additives

375P Evaluation of apparent metabolizable energy of pequi oil in broiler chickens. Murillo N. Carvalho*², Pedro Pereira Leite Trevisani², Jéssica M. Cruvinel^{3, 2}, Beatriz A. de Souza², Felipe F. dos Santos^{1, 2}, Cássio Y. Oura^{1, 2}, Fernanda Kaiser de Lima-Krenchinski^{1, 2}, Priscila M. Groff-Urayama^{4, 2}, Tatiane Souza dos Santos^{3, 2}, Julianna Batistioli^{2, 3}, Erica S. Mello^{2, 3}, José R. Sartori^{2, 3}, Antônio Celso Pezzato^{1, 2, 3}, Amanda B. Cirino²; ¹Animal Nutrition, UNESP, Botucatu, São Paulo, Brazil, ²FMVZ, UNESP, Botucatu, Brazil, ³Animal Nutrition Department, School of Veterinary Medicine and Animal Science, Botucatu, Brazil, ⁴animal breeding and nutrition, São Paulo State University (UNESP), Botucatu, Brazil.

Pequi (Caryocar brasiliense Camb.) is a fruit native to the Brazilian cerrado biome and is considered one of the species of greatest socioeconomic relevance to the region. The present study aimed to determine the apparent metabolizable energy (AME) of pequi oil (PO) in broiler chickens. A total of 90 male broilers (Cobb 500) was placed in metabolism cages (0.4m height x 0.5m length x 0.60m width) at 21 days of age, distributed in a completely randomized design, with three treatments of 6 repetitions and 5 birds/cage. The treatments included i) basal diet - based on corn and soybean meal, formulated to correspond to nutrient requirements that were equal to or slightly lower than those recommended by Rostagno et al. (2017). ii) Soybean oil - introduction of 10% SO in the basal diet. iii) PO - introduction of 10% PO in the basal diet. All cages were equipped with a trays previously prepared and lined with plastic for the collection of excreta. Excreta collection involved an adaptation period of 5 days of adaptation the diets and 5 days of excreta collection (feces and urine) with control of feed intake. Unmarked excreta in the first collection and marked excreta in the last collection were discarded (marker as 1% ferric oxide). There were two daily collections 08:00 am and 4:30 pm and the excreta collected were packed in plastic bags, weighed and stored in the freezer. The excreta was thawed, weighed and homogenized for removal of a sample from each experimental unit (200-300g sample/cage). In sequence, dried in a forced ventilation oven at 55 degrees celsius for 72h, in order to promote pre-drying and air dry matter determination. Furthermore, the samples were ground in a knife mill (16 mesh sieve with 1mm sieves). The samples and diets were sent to the bromatology laboratory for analysis of dry matter and crude energy. Based on the results of the analyzes, the value of AME was calculated using the formula proposed by Matterson et al. (1965). Data were analyzed using the ANOVA procedure of SAS. In these experimental conditions, the crude energy values for soybean oil were 9,379 kcal/kg and for pequi oil 9,112 kcal/kg. The AME of soybean oil was 7,079 kcal / kg, and the pequi oil was 6,664 kcal/kg as fed, and these data showed no difference in the F test (P = 0.081). It is possible to verify that there was a 73% utilization of PO by broiler chickens. Pequi oil is a fruit rich

in bioactive compounds important to animal health, and the determination of AME is the first step in evaluating this ingredient in the diet of birds. This research was financially supported by grant 2018/25363-2 from the São Paulo Research Foundation (FAPESP).

Key Words: metabolizable energy, broiler chickens, pequi, Caryocar brasiliense

376P Effect of *Lactobacillus plantarum* supplement on the growth performance, nutrient digestibility, gas emission, excreta microbiota, and meat quality of broilers. Hyun Ju Park*, Vetriselvi Sampath, Je Min Ahn, Chai Bin Lim, In Ho Kim; *Department of Animal Resource and Science, Dankook University, Cheonan, Korea (the Republic of).*

Poultry production has grown rapidly and becomes a competitive industry all over the world. The growth of poultry production has a profound impact on the demand for nutritious feed ingredients. Probiotics are defined as live microorganisms that are beneficial to the host. The goal of this analysis was to investigate the effects of *Lactobacillus plantarum* (*L. plantarum*) on the growth performance, nutrient digestibility, gas emission, excreta microbiota, and meat quality of broilers. The Animal care and use committee of Dankook University has approved the protocol (No: DK-1-2022) of this trial and birds were treated humanely. A total of 288 Ross308, 1-day-old chicks (mixed sex) with an initial weight of 42.61 ± 0.49 g (mean \pm SD) were procured from Cherry-Buro hatchery and randomly allocated to one of two treatments with 8 replicated cages (18 chicks/cage). For a period of 35 days, control treatment chicks were fed a commercial corn-soybean meal-based (CON) diet, whereas, TRT-1 chicks fed CON with 0.10% *L. plantarum* supplement. *L. plantarum* supplement was commercially obtained from Micro-solution. Co Ltd. (South Korea). It contains 1.2×10^9 colony-forming units (CFU kg⁻¹) of *L. plantarum*. All data were analyzed as a completely randomized block design using the GLM procedure of SAS (version 9.2). Cages were considered as experimental units and significant differences were identified using T-test. Variability in the data was expressed as the standard error of means and P < 0.05 was considered as statistically significant. The dietary inclusion of 0.10% *L. plantarum* supplementation has a trend to significantly increase the body weight gain on days 7, 21, and the overall (p=0.079, 0.011, and 0.037 respectively) trial period compared to the CON diet. In addition, 0.10% *L. plantarum* supplementation has significantly decreased H₂S (p=0.046) concentration. Also, it has significantly increased the *lactobacillus* population ((p=0.041) and reduced the *E. coli* count (p=0.054) compared to the CON diet. However, throughout the trial, there were no significant differences observed on nutrient digestibility of dry matter, nitrogen, and gross energy, as well as meat quality traits in broilers, fed a 0.10% *L. plantarum* diet. In summary, *L.*

plantarum probiotic strain has increased the body weight, *Lactobacillus* population, reduced *E. coli* counts, and H₂S concentration in broilers. Thus, we suggest 0.10% of *L. plantarum* diet could serve as a better alternative feed additive to enhance poultry production.

Key Words: *Lactobacillus plantarum*, growth performance, excreta microbiota, broiler

377P The impact of dietary supplementation of NuPro® in the starter diets on the 21 day growth performance of broiler chickens. Rebecca Delles*¹, Tuoying Ao¹, Marquisha Paul¹, Anthony Pescatore², Mike Ford², Daniel Graugnard¹, Ronan Power¹; ¹Research, Alltech, Inc., Lexington, Kentucky, United States, ²Animal and Food Sciences, University of Kentucky, Lexington, Kentucky, United States.

Healthy chicks and early nutrition are key factors for birds to reach their genetic potential. Nucleotides are semi-essential nutrients in early life development and during times of stress. Nucleotides can be synthesized or recycled through the *de novo* or salvage pathways, respectively. During the first weeks of life these *in vivo* mechanisms may not provide an adequate supply of nucleotides for an organism. NuPro® is a yeast extract that contains 40% crude protein, 8% amino acids, glutamic acid, inositol, and 5-7% nucleotides and may serve as a potential source of dietary nucleotides. The addition of NuPro® to the starter diet may support growth performance by increasing nucleotide availability. The objective of this study was to determine the effect of supplementing NuPro® in the starter period (d1–9) on the growth performance of broiler chickens for 21d. One-day old male Cobb by-product breeder chicks were randomly assigned to either one of two dietary treatments, a corn-soy control diet (CON), or a corn-soy diet supplemented with 2% NuPro®. Both diets had the same nutrient level and chicks were fed respective dietary treatments for 9d, after which all birds were fed the same corn-soy control diet. Eight replicate pens of 24 chicks per pen were randomly assigned to each of the dietary treatments. Chickens were housed in floor pens in an environmentally controlled room with *ad libitum* access to feed and water. Data were subjected to Analysis of Variance (ANOVA) to identify treatment effects, with least square differences (LSD) used to determine mean differences. Chicks fed NuPro® in the starter diet had higher ($P < 0.01$) weight gain (150 vs. 138 g/bird) and a lower ($P < 0.01$) feed to gain ratio (1.24 vs. 1.33) compared to CON. After 21d, birds fed NuPro® had significantly higher weight gain (762 vs. 715 g/bird) and feed intake (1078g vs. 1027g) compared to CON. Based on the results from this study, dietary supplementation of NuPro® in the starter diet positively impacted 21d growth performance of broiler chickens.

Key Words: Broiler chicken, Performance, Nucleotide, Starter Diet

378P Evaluation of a postbiotic as an intervention for mitigating necrotic enteritis in experimentally infected Poult. Sci. 100 (E-Suppl 1)

broilers. Tri Duong*¹, Sara Llamas Moya¹, Cynthia Rasmussen¹, Brett Lumpkins², Greg F. Mathis²; ¹Kerry Group, Plc., Beloit, Wisconsin, United States, ²Southern Poultry Research, Athens, Georgia, United States.

Necrotic enteritis is a multifactorial disease characterized typically by an over-growth of *Clostridium perfringens* and is estimated to have an annual cost to the poultry industry of 6 billion USD worldwide. Although traditionally managed using antibiotics, consumer preferences and regulatory pressures to reduce their use has increased the need to develop interventions to mitigate effects of necrotic enteritis in poultry production. Postbiotics, non-viable preparations of probiotic microorganisms containing microbial metabolites and other cellular material, may be potentially important alternatives to antibiotics in managing necrotic enteritis because of their beneficial health effects. In this study, we conducted two replicate trials to evaluate the effects of the administration of a postbiotic preparation of a probiotic Lactic Acid Bacterium to broiler chickens in an experimental co-infection model of necrotic enteritis. Broilers were fed untreated feed or feed supplemented with either BMD or postbiotic, and necrotic enteritis was induced experimentally using co-infection with *Eimeria maxima* and *C. perfringens*. Uninfected broilers fed untreated feed served as an additional control. Growth performance was evaluated through 28 d post-hatch and gross intestinal lesions were evaluated at 21 d post-hatch. Data from two replicate trials were analyzed using ANOVA and blocked by trial. No significant treatment × trial interactions were observed. Administration of BMD and postbiotic increased BW at 21 d ($P = 0.037$) and 28 d ($P = 0.025$) to levels similar to the uninfected broilers and improved FCR over 0 to 21 d ($P < 0.001$) and 0 to 28 d ($P < 0.001$) when compared to the infected control ($P < 0.001$). Additionally, administration of BMD and postbiotic reduced necrotic enteritis-related mortality ($P < 0.001$) and severity of gross lesions ($P < 0.001$) when compared to the infected control and to levels similar to the uninfected broilers. These data suggest administration of a postbiotic preparation from a probiotic Lactic Acid Bacterium may be used to mitigate effects of necrotic enteritis in broiler chickens.

Key Words: postbiotic, necrotic enteritis, broilers

379P The effect of various levels of a dacitic (rhyolitic) tuff breccia on growth performance of broilers mildly challenged with Eimeria spp. Po-Yun Teng*¹, Janghan Choi¹, Sudhir Yadav¹, Fernanda L. Castro², Jon Ferrel², Woo K. Kim¹; ¹Department of Poultry Science, University of Georgia, Athens, Georgia, United States, ²AZOMITE Mineral Products Inc., Nephi, Utah, United States.

The aim was to investigate the effect of a *dacitic tuff breccia* (DTB) on broilers challenged with a mild *Eimeria* spp. infection. A total of 600 one-d-old Cobb 500 male chicks (44.81g; SD ± 0.26g) were randomly assigned to a complete block design of 5 treatments, 10 replicates of 12 birds for 26d. Birds were housed in cages (0.09 m²/bird) with *ad libitum* water and feed. Treatments

consisted of unchallenged (UC) and challenged (CC) controls (0% DTB), and three challenged groups with 0.125, 0.25, or 0.5% DTB. At d14, CC and DTB birds were orally gavaged with a pool of sporulated oocysts (12,500 *E. maxima*, 12,500 *E. tenella*, and 62,500 *E. acervulina*) and UC received water (1 mL). Corn-soybean meal-based diets were divided into starter (0-14d) and grower (14-26d). At 14, 20, and 26d, average daily gain (ADG), average daily feed intake (ADFI), and gain:feed ratio (GF) were calculated. At d19, intestinal permeability (1 bird/cage) was measured by Fluorescein Isothiocyanate-dextran (FITC-d, 2.2 mg/bird). At d20, intestinal lesion score (4 birds/cage) was assessed. Performance and permeability data were analyzed using GLIMMIX procedure and Tukey's test and lesion score using npar1way procedure and DSCF test in SAS (SAS Institute, Cary, NC; v9.4) with significance at $P < 0.05$. From 0-14d (pre-challenge), no differences were seen in ADG and ADFI ($P > 0.203$). GF was higher in 0.125 (4.47%), 0.25 (3.35%), and 0.5% of DTB (2.89%) than controls (CC and UC) ($P < 0.001$). From 14-20d (challenge phase), ADG and ADFI were the highest in UC (112.12 and 32.47%, UC vs. CC, $P < 0.001$), showing the challenge effectiveness, without differences amongst DTB groups. GF was higher in UC than the other groups (0.686, $P < 0.001$), followed by 0.125% DTB (0.439), 0.25% DTB (0.438), 0.5% DTB (0.429), and CC (0.428). From 20-26d (recovery phase), ADG ($P = 0.05$) and ADFI ($P < 0.001$) were 14.49 and 18.09% higher in UC vs. CC, respectively. Although not significant, 0.125% DTB had the highest GF amongst all treatments during this phase (3.03% and 7.75% over CC and UC, respectively, $P = 0.189$). Overall (0-26d), ADG and ADFI of UC birds were 34.28 and 21.82% higher, respectively, than CC ($P < 0.001$). GF was the highest in UC (12.38% UC vs. CC), and birds fed 0.125 and 0.25% DTB were more efficient than CC (0.644 and 0.639 vs. 0.622, respectively) ($P < 0.001$). Intestinal permeability was lower, thus better, in UC birds than the challenged groups, and 0.125% DTB was better than 0.5% ($P < 0.001$). No differences in lesion scores were observed amongst challenged groups ($P > 0.242$). The mild *Eimeria* spp. infection negatively impacted growth performance, while DTB at 0.125 and 0.25% showed potential in improving GF pre-challenge and during challenge and recovery phases.

Key Words: Dacitic tuff breccia, Coccidiosis, Broiler chickens

380P Effects of dietary vitamin C, vitamin E, and betaine on productive performance, egg quality, and stress marker of laying hens raised under heat stress conditions. Sung Hoon Kwon*, Jeong Hun Nam, Deok Yun Kim, Cha Yeong Lee, Chan Ho Kwon, Seung Yeon Won, Dong Yong Kil; *Chung-Ang University, Anseong-si, Korea (the Republic of)*.

The objective of the present experiment was to investigate the effects of dietary vitamin C (VC), vitamin E (VE), and betaine (BT) on productive performance, egg quality, and

stress marker of laying hens raised under heat stress conditions. A total of two hundred eighty 47-wk-old Hy-Line Brown laying hens were allotted to 1 of 4 dietary treatments with 7 replicates in a completely randomized design. Each replicate had 10 hens. All nutrients and energy in the control diet were formulated to meet or exceed the requirement estimates for Hy-Line Brown laying hens. Three additional diets were prepared by adding 250 mg/kg VC, 500 mg/kg VE, or 3,000 mg/kg BT to the control diet. The experimental diets and water were provided to hens on an ad libitum basis for 8 wk. Average daily room temperature and relative humidity were $30.7 \pm 1.41^\circ\text{C}$ and $72.5 \pm 11.61\%$, respectively. According to heat stress index calculated from the ambient temperature and relative humidity for laying hens, the heat stress index was approximately 82, indicating that hens were raised under heat stress conditions in this experiment. Productive performance including BW gain, egg production rate, feed intake, feed conversion ratio, egg mass, and broken egg production rate was recorded during 8 wk of experiments. Egg quality was assessed using randomly-collected samples of 10 eggs per replicate with 5 eggs per day during the last 2 days of the experiment. Blood samples were collected by selecting one hen per replicate at the end of the experiment. The heterophil to lymphocyte ratio (H:L ratio) in the blood was calculated as a stress marker. The data were analyzed by one-way ANOVA in completely randomized design using the PROC MIXED procedure in SAS. The replicate was used as the experimental unit for all measurements. Significance for statistical tests was considered at $P < 0.05$. Results indicated that hens fed diets containing 500 mg/kg VE had a less ($P < 0.05$) egg production rate than other dietary treatments. However, there were no differences in other productive performance among dietary treatments. For egg quality, hens fed diets containing 3,000 mg/kg BT had a less ($P < 0.05$) eggshell thickness than those fed the diets containing 250 mg/kg VC or 500 mg/kg VE. However, other egg quality including eggshell strength, Haugh unit, egg yolk color, and eggshell color was not affected by dietary treatments. Hens fed diets containing 3,000 mg/kg BT had a less ($P < 0.05$) H:L ratio than those fed diets containing 500 mg/kg VE. In conclusion, dietary supplementation of vitamin C, vitamin E, and betaine has no beneficial effects on productive performance, egg quality, and stress marker of laying hens raised under the current heat stress conditions.

Key Words: Betaine, Heat stress, Laying hen, Vitamin C, Vitamin E

381P Effects of organic acids and essential oils on growth performance, serum biochemistry, antioxidant enzyme activities, intestinal morphology, and digestive enzyme activities in broiler chickens. Janghan Choi*¹, Amit Singh¹, Xixi Chen², Yan Lei³, Woo K. Kim¹; ¹*Department of Poultry Science, University of Georgia, Athens, Georgia, United States*, ²*DDC Nutrition LLC, Walnut, California, United States*, ³*DadHank (Chengdu) Biotech Corp, Chengdu, China*.

This study was conducted to investigate the effects of organic acids (OA) and essential oils (EO) on growth performance, serum biochemistry, antioxidant enzyme activities, intestinal morphology, and digestive enzyme activities as antibiotic alternatives in broiler chickens. A total of 600 one-day-old broiler chicks were randomly allotted to 5 treatments: 1) positive control (PC): a corn-soybean meal diet containing 500 mg/kg bacitracin methylene disalicylate-50 (BMD-50) 2) negative control (NC): PC without BMD-50 3) OA: NC + 2 kg/ton OA 4) EO: NC + 300 g/ton EO 5) OAEO: NC + 2 kg/ton OA + 300 g/ton EO. Each treatment had 6 replicates with 20 birds per pen. This experiment lasted 42 days including 3 feeding stages of starter (D 0 to 14), grower (D 15 to 28) and finisher (D 29 to 42). Growth performance were recorded on D 14, 28 and 42. On D 35, serum and liver samples were collected for analyzing serum chemistry and activities of antioxidant enzymes [(glutathione peroxidase (GPx) and superoxide dismutase (SOD)), respectively. Intestine samples (duodenum, jejunum, and ileum) were also collected for intestinal morphology [villus height (VH), crypt depth (CD), and VH:CD] and jejunal brush boarder enzyme activities (leucin aminopeptidase) assays. One way ANOVA followed by Duncan's tests was performed. In the starter phase, the PC, EO and OAEO groups had significantly lower feed conversion ratio (FCR) compared to the NC group, and the OA group had similar FCR with the PC group ($P > 0.05$). In the grower phase, birds fed PC had significantly higher body weight (BW) compared to the birds fed NC, and birds fed EO had similar BW compared to birds fed PC ($P > 0.05$). While final BW of birds fed OA was similar compared to birds fed PC ($P > 0.05$), FCR of the OA group in the finisher phase was lower than the PC group ($P < 0.05$). No significant differences were observed among treatments in the activities of leucine aminopeptidase, GPx and SOD ($P > 0.05$). Whereas the OA and EO group had increased ileal CD compared to the OAEO group, the OA group had significantly higher VH:CD compared to the EO group. The blood total protein level was significantly higher in the PC group, and the blood globulin and potassium levels of the PC and OA groups were significantly higher than the EO group. Thus, supplemental OA could be an effective antibiotic alternative in broilers as birds fed OA had comparable growth performance and gut health with birds fed an antibiotic.

Key Words: Organic acids, essential oils, broilers, broiler chickens, antibiotic alternatives

382P Comparison of alternative feed additives to antibiotics to prevent subclinical necrotic enteritis in broilers. Masoumeh Ghiyamatiun, Maziar Mohiti-Asli*; *Department of Animal Science, University of Guilan, Rasht, Iran (the Islamic Republic of).*

Necrotic enteritis (NE), caused by *Clostridium perfringens* infection, is one of the most common enteric diseases, causing a major economic impact on the poultry sector worldwide. Nutritional factors, particularly feeding

high quantity of cereals containing non-starch polysaccharides (wheat and barley) enhance disease by increasing digesta viscosity, mucus production and bacterial growth. Animal proteins, particularly fish meal, enhance *C. perfringens* proliferation and toxin production. Although antibiotic growth promoters (AGP) effectively controlled NE in the past, recent trends in reducing AGP use led to an increase in disease outbreaks in broiler flocks. Current approaches to controlling this disease focus on addressing predisposing factors, rather than trying to eradicate the bacteria. The objective of the current study was to investigate the effect of AGP alternatives on broiler performance, gut health and prevention of subclinical NE. A total of 336 one-day-old Ross 308 male broilers were distributed in a completely randomized design with 7 treatments and 4 replicates of 12 birds each. The treatments consisted of: 1) Negative control (NC; corn-soybean meal-based diet, without feed additive), 2) Positive control (PC; diet containing wheat and fish-meal to induce NE, without feed additive), 3) Antibiotic (PC diet + 0.1 g/kg zinc bacitracin), 4) Probiotic (PC diet + 0.4 g/kg Bioplus 2B®), 5) Prebiotic (PC diet + 1 g/kg XPC™), 6) Phytobiotic (PC diet + 0.2 g/kg OXPlant™) and 7) Organic acid (PC diet + 2 g/kg B.I.O. Acid Ultra). Chickens were fed their respective experimental diets from 1 to 42 d of age. Growth performance traits were measured in this period. On days 29 and 41, two birds from each replicate were slaughtered to collect cecal contents for bacterial enumeration and to assess the organ weight. Data were analyzed using GLM procedure of SAS, followed by Tukey's HSD test when effects were $P < 0.05$. Results demonstrated that administration of antibiotic or their alternatives to PC diet did not affect feed intake, average daily gain, feed conversion ratio and organs weight. Subclinical sign of NE was detected in broilers at 41 days of age when cecal bacterial count evaluated. However, broilers fed probiotics, prebiotics or organic acid in the diet had lower count of *C. perfringens* than PC group, there was no significant difference compared with NC. The results indicated that the administration of phytobiotic compared to antibiotic not only reduced the count of *C. perfringens* and Coliforms, but also increased the count of lactobacilli. Therefore, phytobiotic can be a suitable alternative to antibiotic in improving broilers intestinal health and reducing susceptibility to subclinical NE.

Key Words: Broiler, Necrotic enteritis, Phytogenic, Prebiotic, Probiotic

383P Effects of supplementation of a blend of encapsulated organic acids in drinking water on performance, intestinal microflora and morphology of broilers. Motahar Rahnama Ghaleroudkhani, Maziar Mohiti-Asli*; *Department of Animal Science, University of Guilan, Rasht, Iran (the Islamic Republic of).*

Microencapsulation is a process of building a functional barrier between the core and wall material to avoid chemical and physical reactions and to maintain the biological,

functional, and physicochemical properties of core materials. The objective of the current study was to study the effects of adding encapsulated and non-encapsulated organic acids (OA) in drinking water on growth performance, ileal microflora and histology were investigated in broilers. A total of 360 day-old Ross 308 male broiler chicks were divided into 6 treatments, 4 replicates, and 15 chicks per replicate in a completely randomized design. Experimental treatments were included: negative control (without OA, in drinking water); positive control (200 mL of a commercial OA, acidifier 4+); 500 and 1000 mL non-encapsulated blend of OA, 500 and 1000 g encapsulated blend of OA in 1000 L drinking water. The blend of OA was composed of butyric, propionic and acetic acids. Performance parameters were determined by week. At day 42, two broilers were randomly slaughtered from each replication, to measure the intestinal microflora, ileum contents were collected to count *Escherichia coli*, *Lactobacillus* and coliforms and the ileum section was dissected for morphometry. Villi height and width, crypts depth, thickness of lamina propria and tonica muscularis layer were measured microscopically, then the villus height: crypt depth ratio and villi surface area was then calculated. All data were analyzed using GLM procedure of SAS statistical software. Broilers received encapsulated blend of OA in drinking water had higher ($P<0.05$) body weight, average daily gain, and European production efficiency factor and lower ($P<0.05$) feed conversion ratio compared with negative control in whole experimental period, while OA content in the water had no effect on consumption of feed and drinking water. Inclusion of 500 g encapsulated or 1000 mL non-encapsulated blend of OA resulted in lower count of *E. coli* compared with the negative control ($P<0.05$). The count of *E. coli* was decreased linearly ($P<0.05$) in ileum of broilers received graded levels of non-encapsulated blend of OA. Broilers received 500 g of encapsulated OA had lower the count of *E. coli* than other treatments. Broilers received different levels of encapsulated blend of OA had smaller ileum villi width ($P<0.05$). Adding 1000 mL of non-encapsulated OA in drinking water increased ileal crypt depth of broilers ($P<0.05$). It can be concluded that the supplementation of encapsulated OA improved growth performance of broilers more than non-encapsulated OA. Addition of 500 g of the encapsulated blend of OA is caused benefit in growth performance, intestinal microflora and morphology of broilers.

Key Words: Broiler, Organic acid, Microencapsulation, Growth performance, Intestinal microflora

384P BoreOX®, a proprietary blend of botanical extracts designed to optimize the vitamin E feed supplementation in broilers nutrition. Bertrand Medina¹, Ashley Wagner¹, Ivan D. Girard¹, Carl Julien^{*2, 3}; ¹*Probiotech International Inc., St-Hyacinthe, Quebec, Canada*, ²*Centre de Recherche en Sciences Animales de Deschambault (CRSAD), Deschambault, Quebec, Canada*, ³*Animal Sciences, Université Laval, Quebec, Poult. Sci. 100 (E-Suppl 1)*

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Feed supplementation with vitamin E increases antioxidant capacity, essential to cope with higher cellular oxidative stress that may happen with fast-growth broiler line. Instead of increasing vitamin E levels too much, complementary antioxidant agents are more and more integrated in animal feed programs to optimize antioxidant nutrition cost and/or capacities. Naturally, botanicals with high polyphenol content gained interest this last decade and were widely investigated as new antioxidant chain links able to spare and/or strengthen Vitamin E inclusion. However, these new promising pure or crude polyphenol extracts were very often ranged under in vitro conditions which are still far from representing both intestinal and cellular media. Therefore, this study designed on broilers aims to determine any predefined in vitro vitamin E substitution factor of a proprietary and novel blend of high content polyphenol extracts (BoreOX®, Probiotech International). A total of 360 Ross 308 male broiler chickens were allocated to three dietary treatments: basal diet with 50 ppm total vitamin E (E50); basal diet with high levels of vitamin E, 130 ppm total vitamin E (E130); basal diet (E50) +100 ppm BoreOX® (BoreOX). The same 3-phase diets were offered ad libitum to 12 replicates of 10 birds from 0 to 34 days of age. No antibiotics nor anticoccidials were used. Body weights (BW) and feed intakes were recorded on day 9, 20 and 34 and reported per period, as mortality rates. Data were analyzed by a mixed model including treatment as fixed effect and barn section as random effect. The plasmatic oxygen radical absorbance capacity (ORAC) was analyzed from 24 birds per treatment at d20. Mortality rates did not differ significantly between treatments. E130 reduced BW at d9 (216 vs 203 g, $P=0.0040$), but improved feed conversion ratio (FCR) during the grower period (9-20d) (1.39 vs 1.49, $P=0.0053$), compared to E50. BoreOX increased average daily gain from d9-20 (56.3 vs 53.4, $P=0.0263$), compared to E50, and was similar to E130 for all the growth performance monitored parameters as: d20 BW, 828 vs 801 g; d34 BW, 2.37 vs 2.33 kg; grower FCR, 1.40 vs 1.41; finisher FCR, 1.49 vs 1.49; and d9-34 FCR, 1.46 vs 1.46, for BoreOX and E130 respectively. Both E130 and BoreOX increased plasmatic ORAC at d20 (4.49 and 4.40 vs 3.87 mM Trolox equivalent, $P=0.0120$ and $P=0.0320$, respectively), compared to E50. Overall, BoreOX showed the same growth performance and the same plasmatic oxidative capacity as E130. Therefore, the last data in vivo determine that 100 ppm of this tested blend of polyphenol extracts could be used to substitute 80 ppm of vitamin E in broiler feed.

Key Words: Antioxidant, vitamin E, tocopherol, polyphenol extracts, broilers

385P Effect of probiotic additives on growth performance of broilers. Pouyan Malekinezhad^{*1, 2}, Laura Ellestad¹, Nazar Afzali², Seyed Homayoun Farhangfar², Monese Hamidi³; ¹*Poultry Science, University of Georgia, Athens, Georgia, United States*, ²*Animal Science,*

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Research on the use of feeding probiotics to broilers has yielded a wide variety of results, which can be due to differences in production, bacterial strains included, dosage, and delivery method. In this study, effects of several probiotics or synbiotics currently under development (Biorun, Iran) were compared with commercially available probiotics [Bactocell (PB) and Gallipro (PG)] for their ability to influence broiler growth performance and intestinal characteristics. The experiment was a completely randomized design with 8 treatments, each containing 6 replicate pens with 12 chicks/pen. Experimental diets were fed from day (D) one until D42 and included: (1) Control diet with no additives; (2) Probiotic Hyprotect (PHT; 0.35 g/kg); (3) Probiotic Hyprozyme (PHZ; 0.4 g/kg); (4) Synbiotic Hyprotect D+ (SPHT; 2g/10 birds); (5) Synbiotic Hyprozyme D+ (SPHZ; 2g/10 birds); (6) Probiotic Hyprolife (PHL; 0.5 g/kg); (7) PB (0.1g/kg); and (8) PG (0.2g/kg). At the end of each diet phase (D10, 24, and 42), pen weight and feed consumption were determined and average daily gain (ADG), average daily feed intake (ADFI), and mortality-corrected FCR were calculated. At D42, one bird from each replicate pen was randomly selected and after euthanasia, 1g of contents was removed from the ileo-cecal junction for counting live colonies of Lactobacilli, Bacillus, and select pathogens present in farm samples (Enterobacteriaceae, coliforms, *Escherichia coli*, Salmonella). Samples were serially diluted to 10⁻⁴ to 10⁻⁶, and 100 µL of each dilution were plated on MRS media for Lactobacilli, TSA agar for Bacillus, VRBD agar for Enterobacteriaceae, sulfite iron agar for Clostridium, and VRB agar for coliforms. Plates were incubated at 37°C for 3 days. A fragment (0.5 cm²) was collected from the center of the ileum for histology. Data were analyzed with the general linear model in SAS, and means of experimental groups were compared using the Tukey method at a 5% probability level (P≤0.05). Dietary treatment significantly affected ADFI, ADG, and FCR (P≤0.05). Dietary PHZ and SPHZ reduced FI and FCR compared to the control group (P≤0.05). Higher weight gain was seen in the SPHZ treatment compared to the PG and PB groups (P≤0.05). The Lactobacilli count in the ileal contents of birds that received PHZ and SPHZ was significantly higher and counts of coliforms, Clostridium, and Enterobacteriaceae were significantly lower than in other treatments (P≤0.05). Crypt depth in SPHZ-fed birds was higher than in the birds fed the other diets (P≤0.05). In conclusion, broilers fed PHZ and SPHZ had a similar performance to commercial probiotics (e.g., PB), and these could be used in poultry production without a decrease growth efficiency.

Key Words: Broiler, *Bacillus subtilis*, Hyprozyme, Hyprotect, Probiotic

386P Effects of a specific blend of oleoresins of spices as a complementary intestinal aid for conventional raised

broilers. Angel R. Alfonso-Avila^{*3, 1}, Bertrand Medina², Ivan D. Girard², Marie-Pierre Létourneau-Montminy¹; ¹Sciences Animales, Université Laval, Québec, Québec, Canada, ²Probiotech International Inc., St-Hyacinthe, Québec, Canada, ³Centre de Recherche en Sciences Animales de Deschambault (CRSAD), Québec, Québec, Canada.

The inclusion of plant secondary metabolites as alternative feed additives (AFA) in poultry industry is based on their antioxidant, anti-inflammatory, and immunomodulant functional properties. The objective of this study was to evaluate the combined effect of a phenylpropanoid and vanilloid rich oleoresins of spices (ratio 3:1; +OS; Probiotech Inc.) with a conventional antibiotic growth promoter (AGP; avilamycin) program on broilers performance. One-day old Ross 308 broilers (n= 1344) were randomly assigned to one of two treatments distributed in 16 pens of 42 broilers each: 1) AGP (Surmax® 150 g/MT – 10% avilamycin) provided during grower (d12 - 19) and finisher 1 (d20 - 29) most critical phases and 2) +OS added through the full cycle at 35 g/MT. Animals received 4 feeding phases. At d4, a coccidia challenge was completed with 15X Coccivac®-B52 (Merck) dose sprayed on feed. Data collection was performed at day 11, 19, 29 and 34. The data was analyzed using a mixed model including the fixed effect of treatment. Comparisons of means were performed with the Tukey test. Zootechnical parameters such as ADG, ADFI, FCR and BW were not affected by treatments during starter and grower phases. In phase 3 (finisher 1), ADG tended to increase when animals received +OS (107.21 vs 104.81 g/d; P=0.07) compared with AGP. At d34, birds fed +OS tended to have higher final BW (2.27 vs 2.23 kg; P=0.08) compared with AGP. Overall animal performance showed the same tendency for higher ADG with +OS (64.6 vs 64.0 g/d; P=0.06). The apparent ileal digestibility of crude protein tended to increase in +OS treatment (78.1 vs 76.8%; P=0.08). However, plasma uric acid, total protein, and albumin were not affected by treatments. Regarding the caecal short-chain fatty acid concentrations, only butyrate tended to be decreased in +OS (8.92 vs 11.33%; P=0.07). At d22, 16 birds per treatment were necropsied and the probability to have a lesion score of E. Maxima was reduced in broilers fed +OS compared with AGP (25 vs 68%; P=0.02). Litter moisture determined at day 11, 19 and 32 was not affected by treatments. The +OS treatment increased foot pad dermatitis score at d21 (0.156 vs 0.057 P=0.03), while no difference was observed at d32. The utilisation of oleoresins of spices as an AFA tended to improve broiler growth performance in a conventional and sanitary-challenged environment, conditions that may mimic those of commercial farms. However, it remains to be established whether increased antioxidant status and reduced systemic inflammation are part of the mechanisms of action of this spice oleoresin blend.

Key Words: Intestinal health support, Feed additives, broiler, oleoresins of species

387P Bone development of broilers fed chondroitin sulfate and manganese. Julian A. Muñoz^{*2}, Taiane S. Martins¹, Tainara A. Sant'Ana¹, Pollyana L. Garbossa¹, Lenise F. Mueller², Laura B. Ferreira², Caio B. Barbalho², Monica M. da Silva², Cristiane S. Araujo¹, Angélica S. Pereira¹; ¹Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga, São Paulo, Brazil, ²Animal Science, Faculty of Animal Science and Food Engineering, University of Sao Paulo, Pirassununga, São Paulo, Brazil.

Glycosaminoglycans and manganese are important elements in bone formation processes and their participation in diets can assist the structural development of genetically improved broilers that are susceptible to musculoskeletal diseases. We aimed to evaluate the effect of diet chondroitin sulfate (CS) and manganese (Mn) supplementation for broilers to determine how these elements affect their bone development. A total of 1152 Cobb male chicks were 47 days housed and distributed in a completely randomized design, in a 4x3 factorial arrangement: four doses of CS (0.00, 0.06, 0.12, and 0.18%) and three doses of Mn (0, 40, and 80 mg/kg), totaling 12 treatments of eight replicates with 12 birds each. In the slaughter process, the legs of a bird per experimental unit were collected and the tibiotarsus (TT) was removed to determine the area, mineral density, and bone strength by radiography. Furthermore, analyses of the mineral profile, morphological attributes, and histology of the cartilage were performed. The results were submitted to the analysis of variance and the means were compared by Tukey test at 5% probability. The presence of CS and Mn in the diets supplied to broilers did not influence bone mineral content and density, phosphorus and manganese levels in TT, absolute weight, and diaphysis perimeter of TT, nor did they affect the number of chondrocytes in the cartilage. When evaluating the morphological traits of the TT, an effect of the CS levels was observed ($P < 0.05$). The 0.18% CS diet decreased the density of organic mass and the perimeter of the proximal and distal epiphysis of the broilers' TT when compared to the results of the broilers receiving the diets of 0.12% CS and 0.06% CS. There was a difference in the TT areas and lengths ($P < 0.05$), being the greatest results achieved by broilers fed diets of 0.12% CS compared to birds on diets without CS. Regarding the levels of Mn, TT of birds fed diets of 40 mg/kg Mn showed larger areas and increased the percentage of calcium in the bones ($P < 0.05$) when compared to the other levels of Mn. It was found that broilers fed diets of 0 mg/kg Mn and their combinations with 0.0% CS and 0.06% CS showed greater percentages of ash than broilers fed diets of 0 mg/kg Mn and the other levels of CS. For bone strength, the effect was at the 0.12% CS level ($P < 0.05$), with the greatest breaking force being seen in TT birds fed with 0.12% SC and without Mn compared to the resistance of TT from birds fed with 0.12% CS and 80 mg/kg Mn. Therefore, supplementation of 0.12% CS, as well as the inclusion of 40 mg/kg Mn in the broilers' diet can be an alternative to improve the TT quality mainly in the morphological attributes, calcium content, and resistance to breaking.

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Key Words: Bone quality, glycosaminoglycans, minerals, poultry, tibiotarsus

388P Effect of dietary supplementation with *Bacillus amyloliquefaciens* and tributyrin on performance and egg quality in second-cycle Bovans White hens. Gerardo Aguilar-Villarreal^{*1}, Dalia L. Carlos-Mateos², Diego Zárate-Contreras¹, Óscar Vázquez-Mendoza³, Arturo Pro-Martínez¹, Fernando González-Cerón²; ¹Livestock Program, College of Postgraduates Campus Montecillo, Texcoco, State of Mexico, Mexico, ²Department of Animal Science, Chapingo Autonomous University, Texcoco, State of Mexico, Mexico, ³Evonik Mexico S.A. de C.V., Tlalpan, Mexico City, Mexico.

This 8-weeks study was conducted in second-cycle Bovans White laying hens to investigate the effect of dietary supplementation with *Bacillus amyloliquefaciens* CECT 5940 (Ba) and tributyrin (TB) on performance and egg quality variables. A total of 100 hens aged 131 weeks were placed individually in conventional cages. Two diets based on corn and soybean meal were randomly assigned to twenty groups of five cages each: 1) Control, diet with 16% crude protein and 2750 kcal ME kg⁻¹, and 2) Ba+TB, control diet supplemented (per kg of feed) with 0.50 g of Ba (2×10^9 CFU/g), plus 0.25 g of TB. Feed and water were provided *ad libitum*. The Ba and TB were products manufactured by commercial companies [Evonik Operations (Germany), and Perstorp Waspik (The Netherlands), respectively]. Egg production (EGGP, %) and feed conversion ratio (FCR, g/g) were recorded daily. Additionally, 240 eggs were collected from each treatment during the last eight experimental days (24 eggs per replicate) to determine in each egg: Haugh units (HU) and proportions (%) of albumen, yolk and egg-shell. Data were analyzed under a completely randomized design with the GLM procedure of SAS. Means were separated with Tukey test ($\alpha = 0.05$). No differences ($P > 0.05$) were observed between treatments in terms of EGGP (85.88 ± 0.02 vs 84.35 ± 0.02), FCR (2.16 ± 0.05 vs 2.12 ± 0.05), proportion of yolk (29.35 ± 0.33 vs 30.14 ± 0.31) and proportion of egg-shell (9.98 ± 0.18 vs 10.33 ± 0.17). In contrast, Ba+TB hens produced eggs with higher ($P < 0.05$) HU (75.21 ± 0.84) and lower ($P < 0.05$) proportion of albumen (59.52 ± 0.34) comparing to Control birds (71.25 ± 0.87 and 60.66 ± 0.36 , respectively). In conclusion, dietary supplementation with *Bacillus amyloliquefaciens* CECT 5940 and tributyrin induces the production of eggs with higher values of Haugh units in second-cycle Bovans White hens.

Key Words: probiotic, butyric acid, egg, layer hens

389P Meat quality in broiler chickens fed Pequi Oil (*Caryocar brasiliense* camb.). Pedro Pereira Leite Trevisani^{*UG1, 3, 2}, Jéssica M. Cruvinel⁴, Murillo N. Carvalho², Beatriz A. de Souza¹, Evelyn Prestes Brito², Carolina Santos², Iasmin M. Farias², Amanda B. Cirino¹, Andrey Savio², Felipe F. dos Santos¹, Cássio Y. Oura², Fernanda Kaiser de Lima-Krenchinski², Priscila M. Groff-

Urayama³, Tatiane Souza dos Santos², Julianna Batistioli², Erica S. Mello², José R. Sartori¹, Antônio Celso Pezzato¹; ¹FMVZ, UNESP, Botucatu, Brazil, ²Animal Nutrition, UNESP, Botucatu, São Paulo, Brazil, ³Animal Breeding and Nutrition, São Paulo State University (UNESP), Botucatu, Brazil, ⁴Animal Nutrition Department, School of Veterinary Medicine and Animal Science, Botucatu, Brazil.

This study was conducted to evaluate the potential effect of pequi oil (PO) on the parameters of pH, cooking loss (%), drip loss (%), shear force (Newtons), lightness (L*), redness(a*) and yellowness(b*) at breast meat quality. A total of 1,440 one-day-old male broilers (Cobb500) were distributed in a completely randomized design into 48 floor pens (2 m² each). The dietary treatments were formulated to correspond to nutrient requirements that were equal to or slightly lower than those recommended by Rostagno et al. (2017). The treatments consisted of: 1) basal diet + Avilamycin at 0.05 g/kg diet and no PO (AGP-antimicrobial growth promoter); 2) basal diet without antibiotic and PO (control, CON); 3) CON + 1.5 g/kg PO (1.5 PO); 4) CON + 3.0 g/kg PO (3.0 PO); 5) CON + 4.5 g/kg PO (4.5 PO); 6) CON + 6.0 g/kg PO (6.0 PO). Each treatment had 8 replicates with 30 birds per pen. At the end of the feeding trial (42 D of age), meat quality of the right and left *pectoralis major* was evaluated from 2 birds per pen. The pH was determined directly in the center of the right-side breast muscle at 24 h post-mortem (HI8314, HANNA). Meat color (L*, a*, and b*) was measured using a colorimeter (Minolta CR-400). The drip loss (left-side breast) was determined as described by Christensen (2003), and cooking loss determined as described by Honikel (1998). Then, shear force was measured perpendicular to the axis of muscle fibers in five replicates for each sample using a TA-CT3 texture analyzer. Data were analyzed using the PROC GLM procedure of the SAS software (version 9.2) Dunnett's range test was performed to compare the AGP treatment with the other treatments. Linear and quadratic effects of dietary PO inclusion levels were studied using polynomial contrasts, excluding the AGP treatment. Statistical significance was determined at P<0.05. In these experimental conditions, those parameters were similar to those of the broilers fed the AGP diet (P>0.05 by Dunnett's test). The PO levels did not influence (P>0.05) pH 24 h postmortem, L*, or b*. Increasing PO level decreased a*, cooking loss, and shear force quadratically (P<0.05). Nonetheless, the values observed in this study are within the acceptable range for meat characteristics, thus meeting current market requirements. This result is promising since residual limits to the use of AGPs in animal products, and their impact on public health and on the ecosystem have already been discussed. Therefore, investigations for new natural products such as PO are fundamental and necessary in poultry production. Acknowledgments: FAPESP by grant 2018/25363-2 and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) finance Code 001 for financing this study.

Key Words: pequi, phytogetic additive, meat quality,

broilers

390P Effects of diets formulated with a bio-emulsifier based on lysophospholipids on performance, energy utilization and nutrient digestibility of broiler chickens.

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An experiment was conducted to evaluate the effects of a lysophospholipid-based bio-emulsifier (LPL) on performance, energy utilization and ileal digestibility of dry matter (DM), fat and crude protein (CP) of broiler chickens at 21 d. These effects were assessed both in a standard diet formulation and in reformulated diets considering LPL matrix contribution, which was also compared to ME reductions and degummed soybean oil (DSO) or acidulated soybean oil (ASO) as oil sources. A total of 392 Cobb 500 male chicks were allocated in battery cages using a completely randomized design with 8 treatments and 7 replicates of 7 birds each from d 0 to 21 posthatch. Treatments consisted of 6 DSO-based diets: positive control (PC1); PC1 formulated with 500 g/ton LPL (PC1+LPL on top); PC1 formulated with 60 kcal LPL matrix (PC1+LPL60); PC1 formulated with 100 kcal LPL matrix (PC1+LPL100); and two negative controls, NC-60 and NC-100 with reductions of 60 and 100 kcal/kg ME, respectively. Two other diets were formulated with ASO: positive control 2 (PC2) and PC2 formulated with 60 kcal LPL matrix (PC2+LPL60). Bird performance was weekly evaluated from d 0 to 21. From 19 to 20 d, partial excreta were collected to determine AME and apparent total metabolizability of DM and CP. At the end of the experiment, ileal digesta was collected to determine ileal digestibility of DM, CP, and fat as well as ileal digestible energy (IDE). Data were subjected to one-way analysis of variance using the GLM procedure of SAS and when significant means were compared by Tukey test (P < 0.05). Orthogonal contrasts were also conducted. There were no effects between soybean oil sources (DSO and ASO) in any parameter (P > 0.05). Birds fed the diet with LPL added on top presented higher AME and total tract metabolizability of energy and DM as well as ileal digestibility of DM and CP compared to broilers fed NC-60 or NC-100 diets (P < 0.05). Effects of ME reductions and LPL utilization were observed, where broilers fed diets with 60 or 100 kcal/kg ME reductions presented lower (P < 0.05) IDE and energy retention than broilers fed PC1 + LPL60 or PC1 + LPL100. Contrasts revealed that LPL, on top or with matrix, improved (P < 0.05) IDE, ileal digestibility of DM, fat and CP as well as FCR from d 0 to 21 compared to the PC1. In conclusion, a lysophospholipid-based bio-emulsifier improved performance, energy utilization and nutrient digestibility of broilers fed standard or reformulated corn-soybean meal diets.

Key Words: biosurfactant, broiler, digestibility, lysophospholipid, oil source

391P Standardized Natural Citrus Extract (SNCE) dietary supplementation recover zootechnical performances losses due to the reduction of protein and energy levels in broiler chicken's feed. Sekhou Cisse*^{GS 1, 2, 3}, Hoa Bui^{2, 3}, Mathilde Buffière², Mohammed El Amine Benarbia^{4, 3}, David Guilet^{1, 3}; ¹EA 921 SONAS, *Beaucouzé, Maine et Loire, France*, ²R&D, *Nor-Feed SAS, Beaucouzé, Maine et Loire, France*, ³Labcom FeedInTech, *Beaucouzé, Maine et Loire, France*, ⁴R&D, *Nor Feed, Angers, France*.

Introduction: In recent month, the price for main raw material that constitute poultry feed are constantly increasing. As example in France, the French Technical Institute for poultry, rabbit and aquaculture (ITAVI) have recorded a 24,2 % increase of poultry feed from January 2020 to January 2021. In order to cope with these increases, the nutritionist must formulate their feed as accurately as possible with the risk of creating deficiencies and seeing the zootechnical performances drop. Energy and protein are among the most expensive items in a feed formula. They are also the ones that directly impact the performances of the animals. Citrus extract supplementation has demonstrated its positive effect under standard formula conditions on ADG and FCR. The purpose of this study was to assess if feed supplementation with SNCE on a degraded ration in terms of energy and digestible amino acids could help recovers performances loss due to this feed degradation. **Experimental design:** The study was performed in the Bangkok Animal Research Center (BARC) in Thailand. 216 broilers chickens (Arbor Acres) were divided into 3 groups. Each group contained 6 replicates of 12 birds: PC group: Standard diet without supplementation; NC group: Low-cost diet without supplementation; NC-SNCE group: Low-cost diet supplemented with 250 ppm of SNCE. The low-cost diet contained 7% less energy and 5% less digestible amino acids than the standard diet. Birds were reared until day 42 and zootechnical performances were recorded at day 1 and day 42. **Statistical analysis:** Statistical analysis was performed by one-way ANOVA test using R Studio software. Statistical significance was considered at $p < 0.05$. **Results:** Regarding the bodyweight, as expected, birds from NC group had lower final weight (3405 g) than birds from PC group (3488 g). We also observed significant differences between birds from NC group verse those received NC-SNCE (NC: 3405 g Vs NC-SNCE: 3519 g, $p = 0,0431$). Interestingly, no statistical difference was observed between birds receiving standard diet (PC group) compared to birds fed with NC-SNCE diet (3519 g, $p > 0,05$). No statistical differences were also observed between the feed consumption and mortality rate of the 3 groups. However, SNCE supplementation allow to improve the FCR by 5,04 % in groups fed with low-cost diet with no statistical difference (NC: 1,527 Vs NC-SNCE: 1,45). **Conclusion:** According to these data, SNCE supplementation allow to recover birds' performances losses due to energy and amino-acids levels reduction. The observed effects are

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probably linked to a better feed assimilation when SNCE is added. SNCE could be a solution to reduce feed cost while maintaining acceptable performance in broiler chickens.

Key Words: Citrus extract, Low-cost diet, zootechnical performances recovery, broiler chickens, experimental study

392P Effects of methyl sulfonyl methane and sodium sulfate on laying performance, egg quality, and antioxidant capacity for laying hens. Yoo Bhin Kim*^{GS 1}, Sang Hyeok Lee², Hyun Gwan Lee¹, Kyung-Woo Lee¹; ¹Konkuk University, *Seoul, Korea (the Republic of)*, ²Konkuk University, *Seoul, Korea (the Republic of)*.

A feeding trial was conducted to investigate the effect of methyl sulfonyl methane (MSM) and sodium sulfate (SS) as a dietary source of sulfur on laying performance, egg quality, corticosterone level in egg yolk, gut morphology, ileal volatile fatty acids, and antioxidant capacity in laying hens. A total of 144 laying hens of Lohmann brown-lite, aged 73 weeks, were randomly allotted to one of three experimental diets for 12 weeks as follows: basal diet (CON); basal diet + 0.2% MSM (MSM); basal diet + 0.3% SS (SS). Each treatment had 8 replicates with 6 hens per replicate. Data were analyzed by ANOVA using the PROC GLM followed by Duncan's multiple range test in SAS. FCR was low ($P < 0.05$) in MSM-enriched diet-fed laying hens at 12 weeks. Dietary treatments did not affect the percentages of dirty and cracked eggs at any ages. Dietary sulfur did not affect egg quality except for Haugh unit at 4 weeks which was lowered ($P < 0.05$) in the SS group. Elevated villus height: crypt depth ratio was observed ($P < 0.05$) in the SS group compared with the CON group. Dietary sulfur did not affect the percentages of SCFAs in the ileum. However, total antioxidant capacity in liver was increased ($P < 0.05$) in laying hens fed diets rich in MSM or SS. In addition, both MSM and SS groups had low concentration of malondialdehyde in serum compared with the CON group ($P < 0.05$). Similarly, malondialdehyde contents in egg yolks were lowest in the MSM group ($P < 0.05$). In conclusion, dietary MSM can lower feed conversion ratio and improve the antioxidant capacity of aged laying hens.

Key Words: methyl sulfonyl methane, sulfur, laying hen, egg quality, antioxidant capacity

393P Production of omega-3 enriched meat through feeding with dietary soluble flaxseed oil for broiler chickens. Sang Hyeok Lee*^{GS 1}, Yoo Bhin Kim², Hyun Gwan Lee¹, Dong won Lee³, Kyung-Woo Lee¹; ¹Konkuk University, *Seoul, Korea (the Republic of)*, ²Konkuk University, *Seoul, Korea (the Republic of)*, ³Haitnim Bio, *Icheon-si, Korea (the Republic of)*.

This study was carried out to investigate the effect of dietary soluble flaxseed oil (SFO), as a source of omega-3 polyunsaturated fatty acids, on growth performance, fatty acid contents of edible meats and internal organs,

malondialdehyde concentration of edible meats and liver, and serum characteristics in broilers. Dietary SFO, as an omega-3-rich food/feed supplement for humans and animals, was manufactured from flaxseed via solubilized process. It was expected that dietary SFO has effect on growth performance, fatty acid contents, malondialdehyde concentration of tissue, and serum characteristics in broiler chickens. A total of 210 day-old unsexed broiler chicks (Ross 308) were randomly allocated into two dietary treatments: no-added control diet and diet containing SFO of 0.5% for 35 days. Each treatment had 7 replicated with 15 chicks per replicate. Data were analyzed by t-test procedure of SAS. The significance level was pre-set at $P < 0.05$. Dietary SFO did not affect growth performance including body weight, body weight gain, feed intake, and feed conversion ratio in broiler chickens during all experiment periods. A significant increase ($P < 0.05$) in docosahexaenoic acid and total omega-3 fatty acids, but not α -linolenic acid and eicosapentaenoic acid, was deposited in thigh and breast meats on day 35 following the SFO feeding. In addition, dietary SFO significantly increased contents of docosahexaenoic acid in liver ($P < 0.05$). On 35 days, dietary SFO increased ($P < 0.05$) malondialdehyde concentration in liver. However, dietary SFO did not affect concentration of malondialdehyde in breast and thigh meat. SFO group increased ($P < 0.05$) concentrations of high-density lipoprotein cholesterol in serum samples compared with control group on 21 days. In conclusion, the present study suggests that dietary SFO improves contents of docosahexaenoic acid and total omega-3 fatty acids in thigh and breast meat and resulted in positive effects on high-density lipoprotein cholesterol of serum in broiler chickens.

Key Words: soluble flaxseed oil, omega-3 polyunsaturated fatty acid, broiler chickens, growth performance, serum characteristic

394P Effect of dietary bacteriophage on *Clostridium perfringens* in broilers. Hyun Gwan Lee*^{GS 1}, Yoo Bhin Kim¹, Sang Hyeok Lee², Jong Pyo Chae³, Yu Jin Kim³, Kyung-Woo Lee¹; ¹Konkuk University, Seoul, Korea (the Republic of), ²Konkuk University, Seoul, Korea (the Republic of), ³CJ Cheiljedang Co., Ltd, Suwon-si, Korea (the Republic of).

Necrotic enteritis (NE) caused by the gram-positive bacterium *Clostridium perfringens* has emerged as important disease associated with major economic losses in poultry industry worldwide. The objectives of this study were to evaluate antibacterial effect of the bacteriophage, either in coated or freeze dried powdered, against *C. perfringens* challenged broiler chickens. Broiler chickens (n=320) were randomly assigned to 32 pens (n=10 broilers/pen) and subjected to one of 4 treatment groups: 1) unchallenged group (NEG); 2) challenged group (POS); 3) challenged group fed diets added with powdered bacteriophage (POP); 4) challenged group fed diets added with coated bacteriophage (COP). On days 21, 22 and 23, all chicks except for the non-challenged control group were

orally inoculated twice a day with 2 mL of either saline or *C. perfringens* (1.0×10^8 cfu/mL). Data were analyzed by one-way ANOVA using the PROC GLM (version 9.4; SAS Institute Inc., Cary, NC). No NE-associated intestinal lesions were noted in the challenged groups. Villus height to crypt depth ratio was higher ($P < 0.05$) in all bacteriophage-treated, challenged groups compared with POS. *C. perfringens* counts in the cecum were significantly decreased ($P < 0.05$) in the bacteriophage-fed chickens compared with POS. Serum IgA levels were highest ($P = 0.046$) in COP. Collectively, our results suggest that both forms of bacteriophage are effective in inhibiting cecal *C. perfringens* counts. Further studies are warranted to evaluate the effect of dietary coated bacteriophage on gut health in experimental NE broiler model.

Key Words: *Clostridium perfringens*, bacteriophage, broiler chickens, gut health, necrotic enteritis

395P Effect of different dietary levels of fenugreek seeds powder on the growth performance of broiler chickens. Deependra Paneru*^{GS 1}, Guillermo Tellez-Isaias², Walter G. Bottje², Jayant Lohakare¹; ¹University of Arkansas at Pine Bluff, Pine Bluff, Arkansas, United States, ²University of Arkansas, Fayetteville, Arkansas, United States.

This study was conducted to examine the effect of fenugreek seed powder (FSP) as a phyto-genic feed additive on the growth performance in broiler chickens. In total, 160 one-day-old Ross 708 straight-run (unsexed)broilers were randomly divided into four dietary treatment groups with four replicate pens per treatment group (10 birds per pen). The treatments were a control group without FSP or 2.5, 5, and 10 g/kg of FSP in the diets. The basal diet was formulated for starter and finisher periods as per the nutrient recommendations of NRC, 1994. All birds were provided with feed and water for ad libitum consumption for 35 days. Bodyweight was found significantly highest in the control group at day 21 ($P = 0.001$) and day 35 ($P = 0.015$) as compared with the FSP treatments showing linear decreasing effect, but it was not different than the 5 g/kg FSP group at day 35 (BW's were 2187 g, 1960 g, 2067 g, and 1955 g, respectively for the four groups). The body weight was not different among the FSP treatment groups at day 35. Bodyweight gain was also found significantly highest in the control group during the starter phase (day 0 to 21) ($P < 0.001$) and the overall phase (day 0 to 35) ($P = 0.014$) showing a linear decreasing effect, but it was not affected during the finisher phase (day 21 to 35). Feed intake was higher ($P = 0.031$) in the control group only during the starter phase compared to other groups showing linear effects. Similarly, the feed conversion ratio was found significantly improved in the control group only during the starter phase ($P = 0.047$) showing linear effects but was not different during finisher and overall phases compared to other treatments. In conclusion, based on the results, the inclusion of FSP up to 10 g/kg diet decreases the overall performance of the straight-run broilers. A confirmation of

the trial using male birds is currently being evaluated.

Key Words: Broiler, fenugreek, growth performance, phytogetic feed additives, body weight

396P Mycotoxins binder alleviates aflatoxin B1 toxic effects on the growth performance, immunological function and intestinal health of broilers. Yujiao Lai*^{GS}, Meng Sun, Yang He, Jiaqi Lei, Yuming Guo, Bingkun Zhang; *College of Animal Science and Technology, China Agricultural University, Beijing, China.*

Aflatoxin B1 (AFB1) is the most toxigenic subgroup of aflatoxins (AF), secondary metabolites of *Aspergillus* species, which impairs performance, productivity and health of animals. Mycotoxin binders are supposed to be an effective treatment of controlling the toxicity of mycotoxins. This experiment was conducted to evaluate whether a commercial mycotoxins-binder, TOXO™, could effectively alleviate the negative effects of AFB1 on performance, immunological function and intestinal health of chickens. A total of 240 one-day-old Arbor Acres broiler chickens were randomly assigned to 4 treatments using a 2×2 factorial randomized design with 2 mycotoxins binder supplemented levels (0 and 2.0kg/t) and 2 AFB1 challenged levels (0 and 200µg/kg) in basal diets. Each treatment contains 6 replicates with 10 birds per replicate from 0 to 42 d of age. All analyses were performed by the GLM procedure SPSS 16.0 software (SPSS Inc., Chicago, IL) as a 2 × 2 factorial arrangement (2 levels of AFB1 challenge and 2 levels of TOXO supplementation). The data were analyzed by two-way analysis of variance with AFB1 and TOXO as the fixed factors. The results showed that AFB1 challenge impaired growth performance by significantly decreasing BWG in day1-21 (P<0.001) and 1-42 (P<0.05), decreasing FI in day1-21 (P<0.05), increasing FCR in d1-21 (P<0.001) and 1- 42 (P<0.05). Compared to the unchallenged groups, AFB1-challenged broilers showed decreased serum IgA (P<0.05), serum antibody titers to Newcastle disease (P<0.05), sIgA contents of jejunal mucosa at day 21 (P<0.05). On the other hand, AFB1 challenge significantly increased the gene expression of pro-inflammatory factors in spleen at day 21 and liver at day 42 (P<0.05), and significantly decreased claudin-1 gene expression at day 42 and increased claudin-2 gene at day 21 in jejunum of broiler chickens (P<0.05) compared to basal diet group. The supplementation of a mycotoxins-binder increased serum antibody titers to Newcastle disease at day 35, serum ALB at day 42 and GLO at d21, serum IgG and IgM at d21, serum antibody titers to Newcastle disease, and the gene expression of claudin-1 in jejunum at day 21 (P<0.05), and significantly decreased the gene expression of IL-6 in spleen at day 21 and IL-1β in liver at day 42, CYP3A4 gene expression in liver at day 21 of broiler chickens (P<0.05) compared with the non-supplemented birds, regardless of AFB1 challenged or not. Conclusively, 200µg/kg mycotoxins-binder can relieve the toxic effects of AFB1 on broilers by relieving immunosuppression induced by AFB1, inhibiting the activity of CYP3A4, and improving

intestinal health of broiler chickens.

Key Words: aflatoxin B1, mycotoxins binder, immunological function, intestinal health, broilers

397P Effect of dietary folic acid and energy density on immune response, gut morphology, and oxidative status in blood and breast muscle of broiler chickens. Fisayo Akinyemi*^{GS}, Deborah I. Adewole; *Animal Science and Aquaculture, Dalhousie University, Valley, Nova Scotia, Canada.*

Folic acid (FA) is an essential vitamin for many metabolic functions including immune response modulation and antioxidant and can help with the functioning of the gastrointestinal tract. Therefore, the objective of the current study was to determine the effect of increasing levels of FA on blood biochemical parameters, immunoglobulin concentrations, jejunal morphology, immune organ weights, and indicators of oxidative stress in blood and breast muscle of broiler chickens fed normal or high energy density diets. Birds were randomly sorted into eight dietary treatments, consisting of eight replicate pens per treatment. Each pen consisted of 25 chicks. The experiment was designed as a 2 x 4 factorial design consisting of two levels of energy: 1) Normal (NE) and 2) High energy density (HE) and four levels (2.2, 5, 10, and 15 ppm) of supplemental FA. The energy contents in the NE diets were 3,000, 3,100, and 3,200 Kcal/kg for starter, grower, and finisher phases, respectively, while the HE diets were formulated to have 100 kcal/kg more energy than the NE diets. On d 36, 8 birds/treatment were euthanized, and plasma samples and jejunum tissues were collected for blood biochemistry and histological measurements, respectively. On d 42, 8 birds/treatment were euthanized, and plasma and breast muscle samples were collected for the determination of superoxide dismutase (SOD) and malondialdehyde (MDA) and serum samples were collected for immunoglobulins (IgG and IgM) determination. Data were analyzed by SAS 16 GLM procedure. There was no effect of FA or energy density on SOD, MDA, IgG, and IgM. Birds fed HE diets had increased (P<0.05) plasma concentrations of calcium and albumin but reduced (P<0.005) weights of ceca and bursa, compared to those fed NE diets. There was a significant interaction (P=0.0016) between FA and energy density on the bursa weight. Dietary supplementation with 5 and 10ppm FA significantly increased (P<0.05) birds heart weight compared to other treatments. Dietary supplementation of 10ppm FA significantly increased (P<0.005) plasma bile acid concentration. Further analysis on jejunal morphology showed significant FA and energy density interactions for villus height (VH; P=0.0226), width (VW; P<0.0001) and crypt depth (CD; P=0.0332). Among the NE group, birds fed 5-15ppm FA had reduced (P<.0001) VW compared to those fed the basal diet. However, among the HE group, 15ppm FA supplementation resulted in an increased VH (P=0.0317) and crypt depth (P=0.0002), compared to the basal diet. In conclusion, feeding chickens with 2.2ppmFA inclusion level in a standard energy diet and

increasing FA inclusions in a HE density diet could be more beneficial for their intestinal health.

Key Words: Folic acid, energy density, blood biochemistry, jejunal morphology, broiler chickens

398P Influence of dietary inclusion of probiotic on egg quality and well-being during the late laying period in brown hens. Marcos Antonio Nascimento Filho*^{GS 1}, Caio Cesar Ouros¹, Marconi Italo Lourenço-Silva¹, Ana Beatriz de Oliveira¹, Andressa Jacinto¹, Alberto Inoue², Ibiara Almeida Paz¹; ¹*Departamento de Produção Animal e Medicina Veterinária Preventiva, UNESP, Botucatu, São Paulo, Brazil*, ²*Chr. Hansen Animal Health, Valinhos, São Paulo, Brazil*.

A study was conducted to investigate the inclusion of probiotic in the diet of brown laying hens at the end of the production cycle on welfare and egg quality. The experiment was conducted in an experimental poultry house, California-type cages with 288 brown laying hens of 71 weeks of age. Birds were assigned to 3 treatments with 12 replicates (8 birds/each) in a completely randomized design during 63 days (3 production cycles of 21 days). The treatments were: Control; 200 - test diet with 200g/ton of probiotic; 400 - test diet with 400 g/ton of probiotic. The control diet was a corn-soybean with antibiotic as a growth promoter formulated to meet the nutrient requirements of laying hens according to the Brazilian tables and the test diet (antibiotic-free) was supplemented with 1.6×10^9 cfu/g

of *Bacillus subtilis* and 1.6×10^9 cfu/g of *Bacillus licheniformis*. At the last 3 days of each production cycle, 36 eggs per treatment were collected to evaluate egg specific gravity, breaking force (TA.XTPlus Texture Analyzer using a 75 mm diameter punch probe, 1 mm/s speed range), percentage of shell, albumen, and yolk, shell thickness, percentage of egg quality problems in the uterus and shell pigmentation failures. Regarding welfare, birds were evaluated by injuring signs caused by other birds, as well as feather-plucking behaviour, fighting, and fear (hiding under other birds). Data were analyzed by One-way ANOVA and Tukey test ($p < 0.05$). Brown laying hens supplemented with probiotic displayed desirable and calmer behaviour compared to the control group, leading to a reduction in disruptive disorders and cracked egg problems. It evidences a better well-being of these birds as well as improvement in the oviduct quality, by the reduction of dirty eggs and shell pigmentation failures. Furthermore, egg shell quality of the birds in the test groups has also been improved for specific gravity, strength and shell thickness compared to the control, which can be attributed to better intestinal health, which is a decisive factor for egg quality. No difference was observed between probiotic dosage used. Thus, the supplementation of probiotic additive seems to be a great alternative for brown hens during late laying period, improving welfare and egg quality.

Key Words: feed additive, well-being, brown hens, egg quality

Metabolism and Nutrition: General Nutrition

399P Estimation of the optimal metabolizable energy and feed intake that maximizes egg mass and economic margin of commercial laying hens. Elias Salvador Tasayco*, Julio Manuel Narvaez Reyes, Lorenzo Rios Junchaya; *Producción Animal, Universidad Nacional "San Luis Gonzaga", Chincha, Peru.*

Objective: Feed represents around 70% of the cost of egg production and energy is the main component of the cost of feed, so assessing and defining the optimal feed intake and energy contributes to improving the productive and economic response in the egg production. An experimental study was carried out with the objective of estimating the optimal consumption of metabolizable energy (ME) and feed that maximizes the egg mass and economic margin of commercial laying hens. Materials and methods: 240 laying hens of the 55-week-old Lohmann Brown genetic line of uniform weight and size were used. The laying hens were distributed in the study area, following the protocol of a Randomized Block Design (DBA). 5 amounts of feed consumption (g/hen/day) and 5 ME consumptions (Kcal/hen/day) were established as treatments: T-1 (105.84g and 296.36 Kcal), T-2 (111.72g and 312.82 Kcal), T-3 (117.60g and 329.29 Kcal), T-4 (123.48g and 345.75 Kcal) and T-5 (110.00g and 299.75 Kcal). The hens consumed the treatment diets for a period of 12 weeks. The measurements were taken from 2 weeks from the start of the study and from there every week until the end of the period. Each of the treatments had three repetitions as blocks, giving a total of 15 experimental units. The variables of egg production, weight and egg mass, feed conversion rate, energy efficiency, Haugh unit of the egg, margin and economic compensation were evaluated. Statistical analysis: Data were subjected to analyze using one-way ANOVA of general linear model procedure of SAS v 9.1. Differences were considered statistically significant when $P \leq 0.05$. The separation of means was done through the Turkey test. Results: Egg production was significantly higher ($P = 0.0130$) for T-5 with 95.08% compared to T-1 with 91.21%. Egg weight was significantly higher ($P = 0.0032$) for T-4 with 64.58g compared to T-1 with 62.45g. Egg mass was significantly higher ($P = 0.0027$) for T-4 with 61.99g compared to T-1 with 56.97g. Feed conversion was significantly improved ($P = 0.0033$) in T-5 with 1.847 compared to T-4 with 1.992. Energy efficiency was significantly better ($P = 0.0009$) for T-5 with 5035 Kcal / Kg compared to T-4 with 5578 Kcal / Kg. The Haugh unit was not affected. The economic compensation was higher for the T-5 at 6.76% compared to the T-1. Conclusions: It is concluded that a feed consumption of 123.48g and 345.75 Kcal of ME per hen/day maximizes the egg mass response and a feed consumption of 110g and 299.75 Kcal of ME per hen/day maximizes the economic margin and economic compensation in laying hens.

Key Words: metabolizable energy, consumption, egg mass, economic margin, laying

400P Effects of including a porcine intestinal mucosa hydrolysate on first-week performance and profitability in broiler chickens. Elías Salvador*², Luis Lujan², Sergi Segarra¹; ¹R&D Bioiberica SAU, *Esplugues de Llobregat, Barcelona, Spain*, ²Universidad Nacional "San Luis Gonzaga", *Chincha, Peru.*

The first week of life of broiler chickens is a key phase that demands highly digestible nutrients to enhance their development, immunity, intestinal health, and body growth. Given that animal protein sources have a better amino acid profile and include higher levels of available amino acids than those of vegetable origin, and that soybeans contain several anti-nutritional factors, our hypothesis was that replacing the latter by the former could enhance the nutritional value of diets for chicken, especially in early stages. Therefore, the objective of the study was to evaluate the effects on performance and profitability of partially replacing vegetable protein sources by a porcine intestinal mucosa hydrolysate (PIMH) in the diet of broiler chickens. A total of 750 1-day-old male Cobb 500 chickens were randomly distributed into three study groups and received different diets including 0 (Control), 2.5 or 5% PIMH (Palbio 50, Bioiberica SAU, Spain) as replacement for vegetable protein sources from 0 to 7 days of age. Ten repetitions were used per study group resulting in 30 pens in floor rearing (25 chicken/pen). Diets were isoprotein and isocaloric and originally formulated based on corn/soy as main protein sources. Body weight (BW), uniformity (U), feed intake (FI), feed conversion ratio (FCR), protein and energy efficiency ratio (PER, EER), gizzard pH (GpH) and profitability were evaluated. Data were analyzed by ANOVA using the GLM procedure of SAS v 9.1. Differences were considered statistically significant when $P \leq 0.05$. The separation of means was done through the Turkey test. After 7 days, chicken fed with the diet including 5% PIMH reached a significantly higher BW compared to Control (216.94g vs 200.15g; $P=0.0005$). Significantly reduced FI ($P=0.0003$) and FCR ($P=0.0001$) were observed with 2.5 and 5% PIMH, compared to Control. PER was significantly improved with 5%, and EER and PER were significantly higher with 2.5% and 5% PIMH, compared to Control. A favorable statistical tendency ($P=0.0754$) was found in the reduction of GpH with PIMH. The maximum profitability was obtained with 5% PIMH (3.54% increase). In conclusion, this study showed significant beneficial effects of partially replacing vegetable protein sources by PIMH in chicken broilers during the starter phase. More specifically, 5% PIMH allowed a 5.29% reduction of soybean meal without having a negative impact, and led to better performance and profitability. This approach should therefore help optimizing the cost of feed, especially given the current trend of rising prices of soy, and could also allow a dilution of the anti-nutritional factors present in soybean meal.

Key Words: broiler, performance, starter phase, diet,

animal protein

401P Energy values of tilapia byproduct meal for broiler chickens determined using the regression method. Yuri Katagiri Dalmoro*, Vítor Santos Haetinger, Carine Adams, Guilherme L. Godoy, Willian Gräf, Otoniel F. Souza, Catarina Stefanello; *Department of Animal Science, Federal University of Santa Maria, Santa Maria, RS, Brazil.*

Fish byproduct meal has been included in poultry feed formulations as a good quality protein source. Different fish species can be used to obtain fish meal, resulting in variable nutrient composition of fish meal. As Nile tilapia production is increasing in some countries, using the processing waste from this fish industry is sustainable and provides an ingredient for poultry diets. In the current study, the ileal digestibility energy (IDE), apparent metabolizable energy (ME), and nitrogen-corrected ME (ME_n) of tilapia byproduct meal was determined using the regression method. A total of 189 slow feathering Cobb 500 male broilers were fed 3 experimental diets with 9 replicate cages of 7 birds each, in a completely randomized design. Broilers were fed a corn-soybean meal reference diet (RD) and 2 test diets (TD) from d 14 to 21 post-hatch. The TD consisted of tilapia byproduct meal that partly replaced the energy sources in the reference diet at 75 or 150 g/kg, such that equal rations were maintained for all energy-containing ingredients across all experimental diets. Excreta samples were collected twice daily from d 19 to 21, and ileal digesta were collected on d 21. Apparent ileal digestibility of dry matter (DM), nitrogen (N), and amino acids as well total tract metabolizability coefficients of DM, N, and energy were calculated. Data were analyzed using the GLM procedure of SAS. Regressions of the test ingredient-associated IDE, ME, or ME_n intake in kilocalories against kilograms of test ingredient intake for cage of birds was conducted using linear regression following SAS statements. The addition of tilapia byproduct meal to the RD linearly decreased ($P < 0.05$) ileal and total tract coefficients of DM and IDE, ME, and ME_n . No effects ($P > 0.05$) were observed on energy and N digestibility. The addition of increasing levels of tilapia byproduct meal to the RD also decreased linearly ($P < 0.05$) the ileal digestibility of all indispensable and dispensable amino acids ($P < 0.001$). The IDE, ME, ME_n values of tilapia byproduct meal for broilers were 2,983, 3,413, and 2,852 kcal/kg of DM, respectively. In conclusion, the current study showed that broilers can utilize a considerable amount of energy and amino acids from tilapia byproduct meal.

Key Words: broiler, digestibility, fish meal, metabolizable energy, nutrition

402P Effect of beak trimming at hatch and the inclusion of oat hulls in the diet on growth performance of brown-egg pullets from 0 to 5 weeks of age. J. Ben Mabrouk*, N. L. Corrales, N. García, L. Aguirre, Gonzalo Mateos, L. Cámara; *UPM, Madrid, Madrid, Spain.*

The effect of infrared beak trimming (BT) at hatch and the inclusion of oat hulls (OH) in the diet on growth performance of brown-egg pullets was studied from 0 to 5 wk of age. The experimental design was completely randomized with 4 treatments arranged as a 2×2 factorial with 2 beak treatments (intact beak vs. beak trimming at hatch) and the inclusion or not of 3% OH in the diet as main effects. Each treatment was replicated 20 times and the experimental unit was a cage with 10 pullets. The control diet was based on corn and soybean meal and contained 2,960 Kcal AMEn/Kg, 19% CP, and 0.98% digestible Lys. Feed intake, BW gain, BW uniformity, and feed conversion ratio were determined by week and cumulatively. Also, energy intake and energy efficiency (Kcal AMEn/g BW gain) were measured at the same ages. Data were analysed as a completely randomized design using the MIXED procedure of SAS, with BT and OH inclusion as main effects and their interaction. When significant differences were detected, the Tukey test was used to separate treatment means. From 0 to 5 wk of age, no interactions between beak trimming and the inclusion of OH in the diet were detected for any of the variables studied and therefore, only main effects are presented. From 0 to 5 wk of age, beak-trimmed pullets had higher mortality than non-treated pullets ($P < 0.05$) with most of the difference observed during the first week of life. Moreover, no mortality occurred for the last 3 wk of the experiment. Non-treated pullets ate more feed, had higher energy intake and BW gain, and had poor energy conversion ratio than beak-trimmed pullets ($P < 0.01$). BW uniformity was better for the intact beak pullets than for the beak-trimmed pullets ($P < 0.01$). On the other hand, OH inclusion increased feed intake and impaired feed conversion ratio ($P < 0.05$). Several interactions between beak trimming and pullet age on performance traits were detected. From 0 to 4 wk of age, non-trimmed pullets grew faster and had better BW uniform than beak-trimmed pullets. However, for the last week of the experiment, opposite results for BW gain were observed. Also, no difference for BW uniformity between pullets was detected for the last week of the experiment. In summary, beak trimming at hatch reduced pullet growth from 0 to 5 wk of age. The inclusion of 3% OH in the diet increased feed intake but did not affect BW gain or energy conversion ratio of the pullets.

Key Words: beak trimming, BW uniformity, growth performance, oat hulls, pullets

403P Influence of the origin of the beans on the chemical composition, particle size distribution, and color of soybean meal. H. Kadardar, L. Aguirre, G. Fondevila, M. Elkissi, Gonzalo Mateos*, L. Cámara; *UPM, Madrid, Madrid, Spain.*

We studied the influence of the origin of the soybean on the chemical composition, protein quality, particle size distribution (PS), and color of commercial soybean meals (SBM). A total of 50 SBM samples from Argentina (ARG; $n=10$), Brazil (BRA; $n=10$), USA ($n=15$), and South Africa

(SA; n=15) were used. Samples from SA were collected from crushing plants in the country of origin whereas for the other 3 origins the samples were obtained in crushing plants in different European locations or at the arrival of the vessels to European ports. The samples were analyzed for main components (proximal analyses, sucrose, oligosaccharides, CF, NDF, and mineral content), protein quality indicators [urease activity (UA), KOH solubility (KOH), protein dispersibility index (PDI), and trypsin inhibitor activity (TIA)], PS distribution [geometric mean diameter \pm standard deviation (GMD \pm GSD)], and color [L* (luminosity), a* (redness), b* (yellowness)] using Minolta camera. Data were analyzed by the GLM procedure of SAS, with origin of the SBM as main effect. In addition, Pearson correlation (r) analyses were conducted to determine the relation between chemical values and color of the SBM using the CORR procedure of SAS. On 88% DM basis, no differences in CP content were detected among SBM origin. The BRA meals had more NDF, raffinose, and Fe but less sucrose and stachyose than the SA meals, with the ARG and USA meals being intermediate (P<0.001). PDI and KOH values were higher for the USA meals than for the South American meals, with the SA meals being intermediate (P<0.01). TIA tended to be higher for the SA and USA meals than for the South American meals (P=0.06). Particle size uniformity was better for the SA and USA meals than for the South American meals (P<0.05). Also, the BRA meals had lower L* and higher a* values than the SA meals, with ARG and USA meals being intermediate (P<0.001). Independently of SBM origin, the greatest correlations were observed between Fe content and L* (r = -0.527; P<0.01) and the a* (r = 0.293; P<0.05) values of the meals. Also, UA, PDI, KOH, and TIA were negatively correlated with the a* Minolta value (r = -0.429, -0.353, -0.482 and -0.766, respectively; P<0.01). The higher a* values of the BRA meals, might reflect the high acidity of the soil and the greater Fe content of the beans produced in this area. The correlation between a* and the PDI value may reflect that extra heat was applied to the BRA beans at the farm to dry the seeds. In summary, chemical composition, protein quality, PS distribution, and color varied widely among commercial SBM according to bean origin.

Key Words: Chemical composition, color, particle size, protein quality, soybean origin

404P Influence of soybean origin on growth performance of broilers fed a semisynthetic diet with soybean meal as the unique source of amino acids from 18 to 21 days of age. G. Fondevila^{*1}, L. Aguirre¹, Emrah Gungor^{2 1}, D. Baruch¹, V. Bernad¹, Gonzalo Mateos¹, L. Cámara¹; ¹UPM, Madrid, Madrid, Spain, ²Ondokuz Mayıs University, Samsun, Turkey.

An experiment was conducted to study the effect of soybean origin on the AMEn content of commercial soybean meals (SBM). In total, 23 samples from Argentina (ARG; n = 8), Brazil (BRA; n = 8), and USA (n = 7) were collected from different European locations by specialized quality control

personnel and analyzed for main components [CP, ether extract, crude fiber, neutral detergent fiber, sucrose, oligosaccharides, and ash content] and protein quality indicators [trypsin inhibitor activity (TIA), KOH solubility (KOH), and protein dispersibility index (PDI)]. The data were analyzed as a completely randomized design using the GLM procedure of SAS with the country of origin of the soybeans as main effect. On 88% DM bases, the SBM from BRA had more CP and less sucrose and stachyose than the SBM from USA and ARG (P < 0.05). The PDI, KOH, and TIA values were higher for the USA meals than for the South American meals (P < 0.05). In addition, a total of 522 one-day old birds were allotted in groups of 8 to 69 battery cages and fed a commercial crumble diet based on corn-wheat and SBM from 1 to 17 d of age. Then, 23 experimental diets that consisted in the mixing of 53% of a N-free diet (43.9% dextrose, 3.50% soybean oil, 3.60% vitamins and minerals, and 2% cellulose) and 47% of each of the 23 SBM samples studied. From 18 to 21 d of age, 3 replicates chosen at random were fed one of each of 23 experimental diets. Feed intake, BW gain, and feed conversion ratio were determined by replicate. Data on growth performance were analyzed as a completely randomized design with country of origin of the beans as main effect using the MIXED procedure of SAS. Soybean sample within each origin was used as a nested effect for the analysis. At the start of the experiment (18 d of age) BW of the birds were similar for all treatments (806, 816, and 796 g for ARG, BRA, and USA meals, respectively). From 18 to 21 d of the test, feed intake was not affected by diet. Birds fed the USA meals grew faster and had better FCR than birds fed the BRA and ARG meals (P < 0.01). In summary, the origin of the beans affected the chemical composition and protein quality indicators of the resulting SBM. Compared to ARG and BRA meals, the use of USA meals as the unique source of amino acids of the diet increased BW gain and improved FCR of the birds from 18 to 21 d of age. Feed mill managers and nutritionists should take into consideration the origin of the beans when evaluating the nutritional value of commercial SBM.

Key Words: broiler, growth performance, protein quality indicators, soybean origin

405P Metabolizable energy values and metabolizability coefficients of soybean meal from different origins for broilers. Lorrayne M. de Paulo, Alison B. Gouveia, Mihayr M. Jardim, Júlia Marixara S. da Silva, João Marcos M. Batista, Helder F. de Oliveira, Marcos B. Cafe, Jose H. Stringhini*; *Zootecnia, Universidade Federal de Goiás, Goiania, Goiás, Brazil.*

Soybean meal is one of the main ingredients used in poultry feeding. Despite being a source of protein and energy for diets, it is rich in oligosaccharides and non-starch polysaccharides, compounds that are poorly digested by broilers. In view of this, the present experiment was conducted to evaluate the apparent metabolizable energy (AME) and nitrogen-corrected AME (AMEn) values and

the metabolizability coefficients of dry matter (DMMC) and nitrogen (NMC) of four commercial soybean meals. The experiment was laid out in a completely randomized design with five treatments and five replicates of eight Cobb 500® male chicks each. Treatments were as follows: basal diet (BD); BD + 40% soybean meal A; BD + 40% soybean meal B; BD + 40% soybean meal C; and BD + 40% soybean meal D. To evaluate the ingredients, the total excreta collection method was performed in the period from 14 to 21 days of age, in which the energy and metabolizability values were determined. The analyzed variables were subjected to analysis of variance and compared by Tukey's test (5%). There was a significant difference for urease activity and protein dispersibility, whose calculated values were 0.05ab, 0.03a, 0.07b and 0.03a (urease activity) and 37.86a, 33.41a, 26.87b and 33.67a (protein dispersibility) in soybean meals A, B, C and D, respectively. However, there was no difference in KOH solubility, whose mean values were 83.31, 83.87, 79.93 and 87.84 for soybean meals A, B, C and D, respectively. No significant differences occurred for the calculated values of AME (kcal/kg), which averaged 3276.98, 3245.77, 3061.88 and 3118.66 in soybean meals A, B, C and D, respectively. For AMEn (kcal/kg), in turn, significant differences were detected, with calculated values of 2553.67a, 2453.02a, 2057.22b and 2175.30ab in soybean meals A, B, C and D, respectively. In terms of metabolizability coefficients, there were no differences for DMMC (61.12, 64.67, 65.40 and 64.26%) or NMC (52.31, 58.23, 57.57 and 54.71%) in soybean meals A, B, C and D, respectively. In conclusion, differences in quality between soybean meals can influence nutrient metabolizability and energy utilization by broilers.

Key Words: poultry feeding, energy utilization, ureatic activity, protein dispersibility, solubility in KOH

406P Influence of the particle size of the limestone and level of supplemented fat of the diet on the productive performance and egg quality of laying hens from 64 to 67 weeks of age. D. Baruch, A.F. de Juan*, J. Ben Mabrouk, L. Dardabou, N. L. Corrales, Gonzalo Mateos; *UPM, Madrid, Madrid, Spain.*

The influence of limestone particle size and level of supplemented fat of the diet on preference behavior, hen performance, and egg quality traits were studied in brown egg-laying hens from 64 to 67 weeks of age. The diets in mash form were based on wheat and soybean meal and contained 2750 kcal AME/Kg and 16.2% CP and 4.0% Ca. The experimental design was completely randomized with 6 diets arranged as a 3 x 2 factorial with particle size of the limestone (coarse, 2-4 mm; fine, <0.6 mm; and an equal mixture of both sizes) and level of supplemented fat (1.48 vs 3.80%) as main effects. The oil used was soapstocks from the refining soy oil industry. Each treatment was replicated 12 times and the experimental unit was the cage with an individual hen. Egg production, and egg weight were recorded daily and feed intake (FI) weekly. In addition, egg quality was recorded in all eggs produced. Hen preference

was estimated in 8 consecutive days by comparing the geometric mean diameter (GMD) ± geometric standard deviation (GSD) of the feed offered at 6.00 am and the feed remaining in the feeder 2 h to 16 h (depending on the day, with 2 h interval between days) after the feed was offered. The first day of the experiment feed samples (300 g) were offered at 6.00 am (immediately after the lights went on) and 2 h later (8.00 am) a sample of the residual was collected. The second day, a new sample of the original feed was offered at 6:00 am and a sample of the residual was collected at 10:00 am. A similar process was followed for the subsequent days. In fact, for the last day, samples of the residual feeds per each replicate were collected at 10:00 pm (16 h interval). Data were analyzed by MIXED procedure of SAS. Hen performance and egg quality traits were not affected by dietary treatment. The GMD of the residual feeds was smaller for hens fed fine limestone than for hens fed coarse limestone, with hens fed the 50:50 mixture being intermediate ($P < 0.001$). An increase in the level of fat from 1.48 to 3.8%, increased the GMD and decreased the GSD of the original diets. Also, an increase in the level of fat reduced ($P < 0.001$) the percentage of fine particles (<630µm) of the residuals compared with the original feed. Hen preference for ingesting the coarse particles of the feed was greater from 10:00 to 12:00 and from 16:00 to 20:00 than for the other intervals. Hens fed fine limestone and 1.48% of supplemented fat had higher ($P < 0.001$) percentage of fine particles than the rest of the hens at 22:00. It was observed that hens had a preference for coarse particles. An increase of the level of fat in the diet reduces fine particles percentage, increasing their intake by hens.

Key Words: fat level, geometric mean diameter, laying hens, limestone particle size, preference behavior

407P Short-term effect of the energy restriction on the phosphorus metabolism in broiler chickens. Rony Riveros Lizana*¹, Matheus D. Reis², Rosiane d. Camargos¹, Nilva Sakomura¹; ¹*Animal Science, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil,* ²*Animal Science, Unesp, Jaboticabal, São Paulo, Brazil.*

The phosphorus represents a small fraction of the diet that could affect the bird performance under deficiency conditions. However, it is not known the interaction mechanism of P with energy. This study aimed to evaluate the short-term effect of the deficiency of available phosphorus on energy utilization. Sixty broiler chickens with ten old-day, with similar body weight (0.387 ± 0.02), were randomly distributed into four dietary treatments. The treatments consist of a factorial arrangement of metabolizable energy (3100 and 2600 kcal ME/kg) and available phosphorus (0.35 and 0.20% avP). Eight birds per experimental unit were allocated in metabolic cages and fed the experimental diets up to 20-old-day. At the end of the experimental period, the body weight (BW) was recorded. Sequentially, the birds were divided into two groups per treatment. In one group, blood samples were collected and stored in an Eppendorf tube to analyze ionized Ca, Mg, and

P. The other group, birds were de-feathered and stored to further body gross energy analyses. The data were analyzed by ANOVA two-way (2ME*2avP) using Minitab v.20 statistical software. The final body weight was affected ($P<0.001$) by the ME level, 0.953 and 0.845 kg/bird for 3100 and 2600 kcal ME/kg, respectively. An interaction between ME*avP ($P=0.015$) was observed on body weight gain, where the treatment 3100 ME+0.20 avP was higher than 2600 ME+0.20 avP. Also, the gross energy content on the body showed an effect ($P=0.022$) of the ME but no interaction with avP ($P=0.247$). For the mineral blood concentration, no interaction was observed for any parameters. Also, ME and avP no affected the iCa and Mg. The avP level had a significant effect ($P<0.001$) on the P blood plasma concentration. In conclusion, ME and avP reductions affect body weight gain, even though they didn't affect the blood parameters and energy deposition.

Key Words: Available phosphorus, Growing chicks, Energy Metabolism

408P Effect of sesame meal as an alternative to soybean meal and its impact on performance, carcass traits, meat quality, and intestinal morphology of broiler chickens. Pouyan Malekinezhad^{*1, 2}, Laura Ellestad¹, Moslem Bashtani², Soheyla Shabkhan²; ¹*Poultry Science, University of Georgia, Athens, Georgia, United States*, ²*Animal Science, University of Birjand, Birjand, Iran (the Islamic Republic of)*.

Lack of adequate food resources for poultry farming is the most important problem in the development of this industry in most parts of the world. Sesame meal is a protein-rich byproduct of oil extraction from sesame seeds and can contain 34-36% protein. Sesame meal also contains adequate amounts of essential amino acids, especially arginine, leucine, and methionine, and is enriched in unsaturated fatty acids such as linoleic acid, oleic acid, and saturated fatty acids. Effects of different levels of sesame meal as an alternative to soybean meal on growth performance, carcass traits, meat quality, and intestinal morphology of broilers were determined. The experimental diets were based on corn-soybean and treatments contained levels of 0, 25, 50, 75, and 100% of soybean meal replaced with sesame meal. In total, 300 1d-old as-hatched Ross 308 broilers were housed in 25 pens, with 5 replicate pens/diet and 12 birds/pen. Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were measured at the end of each diet phase (days 10, 24, and 42). At the termination of the experiment, ten birds from each replicate (2 birds/pen) were euthanized and subjected to carcass analysis and evaluation of villus height and width, crypt depth, villi height: crypt depth ratio, and goblet cell count in the ileum. Carcass yield, meat cut yield, and meat quality according to water-holding capacity (WHC) and breast level malondialdehyde (MDA) were determined. Data were analyzed using the general linear model procedure of SAS. With up to 50% replacement of soybean meal with sesame meal, no significant differences in FI, BWG, and FCR were

observed as compared to the control treatment with 100% soybean meal. ($p>0.05$). With 75% and 100% replacement of soybean meal with sesame meal, FI and BWG decreased and FCR increased significantly ($p<0.05$). Carcass efficiency and relative weight was not significantly decreased with sesame meal substitution ($P>0.05$). With an increasing percentage of soybean meal replaced with sesame meal, WHC increased and MDA decreased significantly ($p<0.05$). Substitution with 50% sesame meal resulted in the highest villi length and width as compared to other dietary groups ($p<0.05$), while replacement with 100% sesame meal had the lowest villi length and width and crypt depth ($p<0.05$). These data demonstrate that all levels of sesame meal increased meat quality and that up to 50% substitution with sesame meal can be used without significant negative growth effects compared to soybean meal; therefore, sesame meal can be introduced as a suitable alternative for broiler diets.

Key Words: broiler, carcass yields, muscle malondialdehyde, sesame meal, water-holding capacity

409P Evaluation of different particle size analysis methodologies for finely to coarsely ground corn. Joseph P. Gulizia*, Santiago J. Sasia, Susan M. Bonilla, Jose I. Vargas, Leah C. Smith, Jorge L. Sandoval, Charles W. Starkey, Wilmer J. Pacheco; *Department of Poultry Science, Auburn University, Auburn, Alabama, United States.*

In animal feed manufacturing, grinding is one of the most expensive cost centers. There are, however, numerous benefits to grinding feed ingredients to a predetermined particle size, including improved feed mixability, increased surface area for digestive enzymatic activity, higher pellet quality, and reduced feed segregation. The objective of this study was to assess 3 methodologies for particle size analysis as measured by the geometric mean diameter. A 2-pair roller mill (Roskamp Champion Series 900-12) was used to mill corn at 3 different settings: 4-1.75, 4-3, 4-3.6 (top-bottom roller pair). For each roller mill setting, 4 methodologies were used to analyze corn particle size; only corn, corn + sieving aid (0.5 g silicon dioxide), corn + agitators (rubber balls and/or bristle sieve cleaners), or corn + both (sieving aid and agitators). A total of 10 replicate samples (40 samples per setting) were analyzed using the ANSI/ASAE S319.4 "Method of determining and expressing fineness of feed materials by sieving" standard using 13 sieves and a Ro-Tap shaker (model RX-30 W.S. Tyler's Ro-Tap, Mentor, OH). Data were analyzed using the GLM procedure of JMP and means separated by Tukey's HSD with statistical significance considered at $P \leq 0.05$. Corn particle size ranged from 764 to 832, 1378 to 1432, and 2005 to 2076 μm from settings 4-1.75, 4-3, and 4-3.6, respectively. At the finest roller mill setting (4-1.75), including both (sieving aid and agitators) resulted in the lower ($P<0.05$) corn particle size compared to only corn and corn + agitators. At roller mill settings 4-3 and 4-3.6, the inclusion of either sieving aid, agitators, or both did not

influence ($P > 0.05$) the particle size. Inclusion of sieving aid, agitators, or both to the 13-sieve method appeared to influence particle size results only on finely ground corn and as corn particle size increased, less influence of sieving aid and agitators were observed. Therefore, analyzing corn particle size without sieving aid and agitators is accurate when using coarser particles in animal feed. However, the combination of both sieving aid and agitators can provide a more precise corn particle size analysis of finer particles.

Key Words: feed, particle size, corn, roller mill

410P Productive performance of Mexican Creole chickens, 13-20 weeks of age, fed diets with different concentrations of metabolizable energy and crude protein. Miguel Á. Matus-Aragón^{*1}, Arturo Pro-Martínez¹, Josafhat Salinas-Ruiz², Fernando González-Cerón³, Juan M. Cuca-García¹, Eliseo Sosa-Montes³, Sergio I. Mendoza-Pedroza¹, Berenice Hernández-Blancas³, Diego Zarate-Contreras¹, Rosalía Ordaz-Contreras¹; ¹*Genetic Resources and Productivity, Livestock Orientation, College of Postgraduates, Texcoco, Mexico, Mexico*, ²*College of Postgraduates Campus Cordoba, Cordoba, Veracruz, Mexico*, ³*Department of Animal Science, Chapingo Autonomous University, Chapingo, State of Mexico, Texcoco, State of Mexico, Mexico*.

The Mexican Creole chickens (MXCC) provide meat to the population of rural areas of the country, they are an unexplored genetic resource and their nutritional requirements are unknown. The objective of this study was to evaluate the productive performance and carcass yield (CY) of the MXCC, from 13 to 20 weeks of age, fed with diets with different concentrations of metabolizable energy (ME, kcal) and crude protein (CP, %). One hundred and ninety-two unsexed chickens were housed in individual cages and were randomly distributed in four experimental diets (48 birds per diet) with a constant ME/CP ratio of 150. ME/CP: 3000/20, 2850/19, 2700/18 and 2550/17. Cumulative productive performance from 13 to 20 weeks of age was estimated in terms of feed intake (FI, g), body weight gain (BWG, g) and feed conversion (FC, g/g). At 20 weeks of age, 16 chickens were randomly selected per treatment and sacrificed to determine the CY (%) and breast yield (BY, %). Data were analyzed as a randomized block design with repeated measures using the GLIMMIX procedure of SAS. Starting weight was included as a covariate. The means were compared with the LSD method at a significance level of 5%. A higher FI ($p < 0.05$) was observed in the diet 2550/17 (6086 ± 155 g), compared to the other treatments: 2700/18 (5745 ± 155 g), 2850/19 (5401 ± 155 g) and 3000/20 (5263 ± 155 g). The FC was lower ($p < 0.05$) in the treatments 3000/20 (7.31 ± 0.26) and 2850/19 (7.38 ± 0.26) compared to the diets 2700/18 (8.07 ± 0.26) and 2550/17 (8.21 ± 0.26). No differences were observed ($p \geq 0.05$) in BWG, body weight at 20 weeks of age (BW20), CY, and BY (global means: 746 ± 24 g, 1923 ± 30 g, 65.58 ± 1.2 %, and 24.16 ± 0.98 %, respectively). In conclusion, the results suggest that any evaluated ME/CP

relationship leads to an equivalent response in terms of BWG, BW20, CY and BY; however, FC is better in diets with a higher concentration of ME and CP, so in practice the cost of the diet must be considered.

Key Words: Mexican Creole chickens, Metabolizable Energy, Crude Protein, Productive Performance, Carcass yield

411P Determination of nitrogen corrected true metabolizable energy content of feed ingredients for poultry diets by Near Infrared Reflectance Spectroscopy. Mary Cope*, Adam J. Davis; *Poultry Science, University of Georgia, Athens, Georgia, United States*.

Near infrared reflectance spectroscopy (NIRS) is a rapid method of analysis that enables a multi-purpose analyzer to be calibrated to predict the nutritional component values of feed ingredients by analyzing their reflectance properties in the near infrared spectrum. The goal of the current research was to create a NIRS calibration curve that accurately predicting the nitrogen corrected true metabolizable energy (TME_N) value for individual feed ingredients. Adult Single Comb White Leghorn roosters were used to determine the TME_N values of feed ingredients. Roosters (8 to 10 per feed ingredient tested) were fasted for 24 hours prior to being precision fed a fixed amount of each feed ingredient. Excreta was then collected for the following 42 hours from the fed roosters and from a group of unfed roosters, which served as controls to account for endogenous losses. Gross energy and total nitrogen content of the feed ingredients and dried collected excreta were determined to calculate TME_N values for each tested feed ingredient. A wide range of determined TME_N values (658 – 5,588 Kcal/kg), and sample variety, from ingredients commonly used in an industry setting like corn, meat and bone meal, and soybean meal, to experimental/alternative ingredients such as black soldier fly larva meal, Jatropha meal, and ground cassava leaves, were obtained. A full near infrared spectral analysis was completed on a ground sample of each feed ingredient using a Bruker MPA: FT-NIR Spectrometer equipped with OPUS Version 7.5 software. A calibration was made for TME_N using 49 feed ingredient samples which resulted in a prediction equation with a correlation coefficient (R^2) of 0.97. This prediction equation was then validated with 316 different feed ingredient samples, and the R^2 between the NIRS and rooster bioassay TME_N values was 0.92, but only 60% of the predicted values were within plus or minus 5% of their actual bioassay determined value. In contrast, subsequent calibration curves based on individual ingredient type, such as corn or bakery meal, resulted in validations in which 100 and 93% of samples, respectively, were within plus or minus 5% of their bioassay determined value. The results indicate that NIRS has the capability of accurately predict the TME_N values of poultry feed ingredients, which would reduce the need for expensive and time consuming rooster bioassays for determining TME_N values on feed ingredients, and enable poultry

nutritionists to formulate more precise diets based on TME_N values of the ingredients actually delivered to the feed mill.

Key Words: Poultry production, Poultry diets, Feed mills, Animal bioassay, Diet formulation

412P Evaluation of combinations of a butyric acid-based product and cranberry pomace extract on the growth performance of mixed-sex broiler chickens. Bruce Rathgeber^{*1}, Joshua Gong², Moussa Diarra², Janice MacIsaac¹; ¹*Animal Science and Aquaculture, Dalhousie University, Brookside, Nova Scotia, Canada,* ²*Guelph Research and Development Centre, Agriculture and Agri-Food Canada, Guelph, Ontario, Canada.*

Feed ingredients with bioactive components that influence gut microbiota have been the subject of developing strategies for maintaining growth performance and health of broiler chickens in the absence of dietary antibiotics. It has been established that an increase of butyric acid in the digestive tract of broiler chickens can change the profile of gut microbiota and provide a protective effect against debilitating intestinal disease. In this study a second dietary ingredient based on extracts from cranberry pomace (CranPo) was combined with a butyric acid- based product (Proformix 650) in effort to enhance bird performance and gut health. The trial was designed as a one-way analysis with supplement as the main effect (no supplement, antibiotic (BMD), Proformix (0.6 kg/tonne starter diet, 0.4 kg/ton grower and finisher diet), Proformix + 0.5% CranPo, Proformix + 1% CranPo, Proformix + 1.5% CranPo). A total of 1104 day old mixed sexed chicks were placed in 48 floor pens. Feed consumption and weight gain were measured from 0-14, 15-24 and 25-35 days of age. Data was analysed by Proc Mixed procedure of SAS. There was an interaction ($P < 0.05$) between dietary treatment and age of the birds on bird body weight. At 14-d of age, birds fed the non-supplemented diet weighed less (342 g/bird) ($P < 0.05$) than birds fed the antibiotic (388 g/bird). At 24-d of age, birds fed the non-supplemented diet weighed less (893 g/bird) ($P < 0.05$) than birds fed the diets containing the antibiotic (1040 g/bird), butyric acid alone (988 g/bird) and Proformix + 0.5% CranPo. At 35-d of age, birds fed the following diets, the non-supplemented diet, the Proformix + 1% CranPo and Proformix + 1.5% CranPo (1908, 1981 and 1963 g/bird, respectively) weighed less ($P < 0.05$) than birds fed the antibiotic (2155 g/bird). There was an interaction ($P < 0.05$) between dietary treatment and age of the birds on feed consumption of the birds. During the starter period, feed consumption was not affected ($P > 0.05$) by dietary treatment. For the grower period, birds fed the non-supplemented diet consumed less feed (83.4 g/bird/day) ($P < 0.05$) than birds fed antibiotic (96.4 g/bird/day) and Proformix + 0.5% CranPo (90.4 g/bird/day). Feed conversion was not affected ($P > 0.05$) by dietary treatment for the duration of the trial. Percent mortality was very low for the trial (2.3%) and was not affected ($P > 0.05$) by dietary treatment. Results from this study indicated there was no

synergistic improvement from combining a butyric acid product and a cranberry pomace extract for broiler growth performance.

Key Words: Broiler, Growth performance, Cranberry, Butyric acid

413P Growth performance and the tibia bone ash of broilers fed various inclusion levels of a black soldier fly larvae meal. Munene Kithama^{* GS 1, 2}, Michael Fruci³, Ed Topp³, Elijah Kiarie⁴, Moussa Diarra²; ¹*Animal Biosciences, University of Guelph, Guelph, Ontario, Canada,* ²*AAFC, Guelph Research and Development Centre, Guelph, Ontario, Canada,* ³*AAFC, London Research and Development Centre, London, Ontario, Canada,* ⁴*Animal Biosciences, University of Guelph, Guelph, Ontario, Canada.*

Insects are receiving great attention as a potential source of protein and or other functional compounds for poultry. The objective of this study was to evaluate the dose response of commercial black soldier fly (*Hermetia illucens*) larvae meal (BSFLM) on growth performance and tibia ash in broiler chickens fed a corn and soybean meal diet. A 35-day trial was conducted using 480 d old male Ross x Ross 708 chicks placed in metabolic cages (10 chicks per cage) and allocated to six experimental diets ($n=6$). The diets were: a corn-soybean meal (C- SBM) based diet with bacitracin methylene disalicylate (BMD) to mimic industry practices, C-SBM without BMD, and C-SBM with replacement of the SBM by BSFLM at an inclusion level of 12.5% (25-32% SBM), 25% (20-26% SBM), 50% (10-13% SBM), and 75% (0% SBM). All diets were prepared in mash form and delivered in a three phase program: starter (d 0-14), grower (d 14-28) and finisher (d 28-35). Body weight (BW), feed intake and mortality were monitored at each phase for calculation of BW gain and feed conversion ratio (FCR) while tibias were collected on d-35. A one-way ANOVA with treatments as fixed effects was run to evaluate the four inclusion levels of BSFLM on growth performance and tibia of broilers, and compare with birds raised with BMD (RwithAb) and without BMD (RwithoutAb). Statistical significance was set at a P -value ≤ 0.05 . The 12.5% BSFLM and 25% BSFLM had a comparable effect with the RwithAb on d-14 BW of the birds ($P < 0.0001$). A similar effect was noted for the BW gain at d-14 ($P < 0.0001$). FCR was lower ($P = 0.0161$) in the RwithAb but the 12.5% BSFLM had a better feed efficiency than the RwithoutAb. There was no difference between treatments for the tibia dry matter and the percentage of tibia weight ($P = 0.5875$). Similarly, there was no significant difference between 12.5% BSFLM and the RwithAb for the tibia ash and percentage weight ($P = 0.1412$). In conclusion, this study demonstrated that feed supplementation with BSFLM at 12.5%, could induce beneficial effects on performance comparable to those of bacitracin during the starter period. Future work investigating lower inclusion rates are warranted as higher BSFLM levels beyond 12.5% appears to have negative effects on bird growth performance.

Key Words: *Hermetia illucens*, Black Soldier Fly, Bacitracin, Soybean meal, Broilers

414P Effect of corn particle size on bulk density, angle of repose, and pellet quality. Susan M. Bonilla*^{GS}, Joseph P. Gulizia, Santiago J. Sasia, Jose I. Vargas, Wilmer J. Pacheco, Charles W. Starkey; *Poultry Science, Auburn University, Auburn, Alabama, United States.*

In broiler diets, particle size of corn can influence pellet quality, bulk density as well as ingredient and feed flowability. The objective of this study was to evaluate the effect of corn particle size on pellet quality, bulk density, and angle of repose, which is used as an indicator to assess flowability. Corn was ground using a 2-pair roller mill to obtain 3 corn particle sizes (832, 1432 and 2250 μm). Diets were steam conditioned at 77°C with a retention time of 45 s and pelleted through a 4.0 pellet die using a pellet mill. Pellets were cooled with ambient air using a counter-flow pellet cooler. Pellet durability index (PDI) was measured using the tumbling box (ASABE method S269.5) modified with the addition of 5 hex-nuts and the Holmen NHP 100 (TekPro Ltd, Norfolk, UK) for 60 seconds with and without sieving the sample after removing it from the test chamber. Bulk density was determined using a quart test weight cup that met Grain Inspection, Packers and Stockyards Administration (GIPSA) specifications. Sample was dropped into the bulk density cup, excess sample removed, and the weight recorded. Angle of repose was measured using a 30.48 cm high x 30.48 cm long x 2.54 cm wide transparent box and a funnel with an opening of 3.18 cm used to transfer samples to the box. Data were statistically evaluated using the GLM procedure of JMP and means were separated by Tukey's HSD with a statistical significance considered at $P < 0.05$. Corn ground to 832 μm resulted in lower ($P < 0.05$; 626.16 kg/m^3) loose bulk density compared to corn ground to 1432 and 2250 μm (686.55 and 676.94 kg/m^3). Corn ground to 832 μm had higher ($P < 0.05$) angle of repose compared to corn ground to 2250 μm (31.25 vs. 29.78°). Diets containing corn with an average particle size of 832 μm resulted in higher ($P < 0.05$; 83.42%) PDI compared to diets containing corn ground 1432 and 2250 μm (80.27 and 79.56%) when PDI was analyzed with the tumbling box. The results of Holmen NHP 100 tester showed that in samples without sieving, the particle size of corn did not influence PDI likely because corn particles larger than 1.58 mm remained on top of the testing chamber and were counted as whole pellets. However, in sieved samples, diets containing corn with 832 μm resulted in higher ($P < 0.05$; 46.31%) PDI compared to diets containing corn ground to 1432 and 2250 μm (39.68 and 36.18%). Reducing corn particle size improved PDI, but reduced its bulk density and flowability.

Key Words: particle size, flowability, angle of repose, bulk density, pellet durability index

415P Assessing dietary corn particle size influences on broilers grown from 1 to 21 days of age. Gabrielle

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In poultry feeding, corn grinding is a significant feed milling cost. As such, the particle size of corn included in broiler diets has been widely discussed. This research evaluated the influence of varying corn particle size (CPS) included in a broiler starter diet on body weight (BW), feed consumption (FC), feed conversion (FCR), and organ parameters. Three CPS treatments [832, 1432, and 2036 μm CPS], with 6 replicates per treatment, were fed to 288 male Cobb 700 broilers (96 birds/treatment) during a 21-d battery cage grow out. Body weight and FC data were collected at 7-d intervals. On d 21, 6 birds/cage were euthanized to assess crop, proventriculus, gizzard, and ceca weights. Data were analyzed as a completely randomized design using the GLM procedure of SAS. Means were separated using Tukey's HSD and significance level was assessed as $P \leq 0.05$. Overall, there were no significant treatment effects for BW at d 7 ($P = 0.918$), 14 ($P = 0.670$), and 21 ($P = 0.722$) or d 0 to 7 ($P = 0.185$), 0 to 14 ($P = 0.155$), and 0 to 21 ($P = 0.463$) FC. There were treatment effects for FCR between d 0 to 7 ($P = 0.019$), with birds on the 2036 μm CPS treatment having an elevated FCR (0.86 g:g) compared to birds on the 832 μm (0.82 g:g) treatment. Treatment differences only approached significance for d 0 to 14 ($P = 0.055$) and d 0 to 21 ($P = 0.052$) FCR, with the highest FCR for both periods associated with the largest CPS. There were no treatment effects for crop, gizzard, or ceca data (absolute weight and g/kg of BW, $P > 0.05$). Proventriculus absolute weights approached significance among treatments ($P = 0.064$), with the largest proventriculus weight reported in birds consuming the smallest CPS. This tendency did not translate to relative (g/kg of BW) proventriculus weights ($P = 0.206$). Based on these results, varying CPS in broiler diets during the early phase of the grow out showed little influence on performance or organ weights. There is some indication that increasing CPS can negatively affect early FCR and smaller CPS may be associated with an enlarged proventriculus.

Key Words: broiler, corn particle size

416P Conditioning temperature directly affects pellet quality. Santiago J. Sasia*^{UG}, Joseph P. Gulizia, Susan M. Bonilla, Jose I. Vargas, Wilmer J. Pacheco; *Department of Poultry Science, Auburn University, Auburn, Alabama, United States.*

During pelleting small feed particles are agglomerated into larger particles, which helps to improve bird uniformity, nutrient digestibility and broiler performance. Pellet quality is the capacity of pellets to resist disintegration and abrasion during handling and transportation. Although there are several factors that influence pellet quality; the objective of this study was to evaluate the effect of 3 conditioning temperatures (75, 80, and 85°C) on pellet quality. A corn-

soybean meal-based diet with 3.42% soybean oil addition was mixed in a twin shaft mixer and then steam conditioned and pelleted using a constant production rate of 636 kg/h using a 4.0 mm pellet die. Pellet samples were collected and immediately cooled. Pellet durability index (PDI) was analyzed using the tumbler method (ASABE method S269.5) modified 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 HSD with statistical significance considered at $P \leq 0.05$. Increasing conditioning temperature improved ($P < 0.05$) PDI when measured with both methodologies. Diets conditioned to 85°C had higher ($P \leq 0.05$; 87.14%) PDI than diets conditioned to 75 and 80°C (82.33 and 84.02%) when PDI was analyzed using the modified ASABE method S269.5. In addition, diets conditioned at 75°C resulted in lower ($P \leq 0.05$; 32.37%) PDI than diets conditioned to 80 and 85°C (48.75 and 64.41%) when PDI was analyzed with the Holmen NHP 100 for 60 seconds. The results of this study confirmed that increasing conditioning temperature improves pellet quality of corn-SBM diets when other factors such as production rate and mixer fat addition remained constant.

Key Words: conditioning temperature, Holmen, tumbler, pellet durability

417P No presentation materials submitted.

418P Effects of maternal fish oil on adipose development in the broiler chick embryo. Minjeong Kim*^{GS 1}, Usuk Jung¹, Suchita Das¹, Lindsay Brown², Shawn Campagna², Jeanna Wilson³, Brynn H. Voyl¹; ¹*Animal Science, University of Tennessee, Knoxville, Tennessee, United States*, ²*Chemistry, University of Tennessee, Knoxville, Tennessee, United States*, ³*University of Georgia, Athens, Georgia, United States*.

Broilers waste feed by converting it to excess adipose tissue. We previously demonstrated that fat accretion in broilers can be reduced by supplementing the broiler hen diet with fish oil (HFO), a source of the long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3). Our objective here was to characterize the effects of HFO on adipose development in broiler embryos. Broiler-breeder (Cobb 500) hens were fed diets containing 3% fat from FO or soybean oil (SO, n-6 PUFA control) for four weeks. Fertilized eggs were incubated to ages E12, E14, E16, E18, and E20. Yolk and fat pads (parafemoral) were snap-frozen for fatty acid profiles and RNA (fat pads). At E16, adipose tissue was also used to isolate preadipocytes for primary culture and assay of adipogenic potential *ex vivo*. Adipose differentiation was quantified by staining cells with a lipid-sensitive dye (Oil Red O) after inducing differentiation. Additionally, lipid and DNA content were visualized with

AdipoRed (Lonza, PT-7009) and NucBlue (ThermoFisher, R37605) staining, respectively, and relative fluorescence intensity of lipid content was normalized to DNA. Targeted RNAseq was used to profile expression of a panel of 504 genes that broadly query adipose development, differentiation, and metabolism. Diet effects were evaluated using Student's t-test ($p < 0.05$). Quantitation of fatty acid profiles (by GC-MS) revealed that HFO significantly enriched yolk and adipose in EPA and DHA and their metabolites at all ages. Preadipocytes isolated from HFO and induced to differentiate *in vitro* accumulated significantly less lipid ($p < 0.01$) than HSO controls, consistent with inhibition of adipogenesis by FO. In addition, HFO preadipocytes exhibited a significant increase in mitochondrial activity, suggesting enhanced oxidative metabolism. Only modest effects of diet on gene expression were observed at E14, when adipose tissue begins to appear in the embryo. By E16 and E20, however, 68 and 71 genes, respectively, were differentially expressed between HFO and HSO (FDR $p < 0.05$), many of which differed at both ages. These genes included key regulators of adipogenesis, metabolism, and stem cell commitment. Several genes that were affected by HFO in embryonic adipose tissue were also differentially expressed after hatch in our previous studies, suggesting that HFO in the embryo induces sustained molecular effects in adipose tissue. In conclusion, these data further support the potential to stably modify adipose accretion in broilers through dietary developmental programming. Additional measures to characterize the mechanisms of programming by maternal fatty acids are underway.

Key Words: adipose, developmental programming, omega-3, broiler, embryo

419P Body weight and uniformity, organ development and jejunal histomorphology in broiler breeder pullets fed n-3 fatty acids enriched diets from hatch through to 22 weeks of age. Aizwarya Thanabalan*^{GS 1}, Elijah Kiarie²; ¹*Animal Biosciences, University of Guelph, Guelph, Ontario, Canada*, ²*Animal Biosciences, University of Guelph, Guelph, Ontario, Canada*.

Enrichment of dietary long chain polyunsaturated n-3 fatty acids (n-3 FA) may be beneficial to broiler breeders (BB) during rearing. So, the effects of feeding sources of docosahexaenoic acid (DHA) and α -linolenic acid (ALA) from hatch through to 22 weeks of age (woa) on growth, organ weight and jejunal histomorphology were investigated. A total of 588-day old Ross x Ross 708 BB were reared on one of three diets: 1) control (CON), corn-soybean meal diet, 2) CON + 1% microalgae (DMA, *Aurantichytrium limacinum*), source of DHA and 3) CON + 2.50% co-extruded full fat flaxseed and pulse mixture (FFF, 1:1 wt/wt), source of ALA. Diets DMA and FFF had similar total n-3 and n-6: n-3 ratio. Diets were allocated to floor pens (28 birds/pen) to give 9 or 6 replicates per diet for CON or DMA and FFF, respectively and fed according to breeder curve in three phases: starter (0-4 woa), grower (5-19 woa)

and pre-breeder (20-22 woa). Individual body weight (BW) was taken weekly, and six birds/pen necropsied at 5 and 12 woa for gastrointestinal, spleen, bursa, and liver weight and samples for jejunal histomorphology. Treatments were fixed effects and all analysis were run using a one way ANOVA, where results were significant at $P < 0.05$. There was no ($P > 0.05$) interaction between phase and diet or diet effect on growth and organ weight. With exception of 5 woa, pullets fed DMA showed ($P < 0.001$) lower BW CV than pullets fed control between 2 and 7 woa. However, pullets fed DMA had higher BW CV at 20 woa than birds

fed either control or FFF. At 5 woa, birds fed DMA had taller ($P \leq 0.01$) villi and deeper crypt than birds fed either CON or FFF but VH or CD were similar ($P > 0.05$) between CON and FFF pullets. At 12 woa, birds fed FFF had taller VH than birds fed CON diet but similar ($P > 0.05$) to birds fed DMA. Different responses to sources of omega-3 FA may implicate other components, however, the BW uniformity and intestinal histomorphology responses suggested benefits of feeding omega-3 FA during rearing.

Key Words: broiler breeder, pullet development, histomorphology, n-3 PUFA

Metabolism and Nutrition: Vitamins and Minerals

420P The mechanism on cadmium-induced autophagy in follicular granulosa cells of laying hens and ameliorative property with selenized yeast. Yuxuan Jiang*^{UG}, lang Li, Jingping Song, Cai M. Wu; *Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu, Sichuan Province, China.*

High residues of cadmium (Cd) in eggs affect egg safety and endanger the health of laying hens. The objective of this trial was to investigate the mechanism of cadmium-induced autophagy in follicular granulosa cells of laying hens and the ameliorative effect of selenized yeast (Se). A total of 64 Lohmann pink-shelled hens (63-wk-old) were randomly divided into 4 treatments with 8 replicates of 2 hens each. The treatments were as follows: control group (CON): corn-soybean meal basal diet; Se group: the basal diet supplemented with 0.4 mg/kg Se from selenized yeast; Cd group: basal diet supplemented with 25 mg/kg of Cd from cadmium chloride (CdCl₂); Se + Cd group: Cd group supplemented with 0.4 mg/kg of Se from selenized yeast. Laying hens were given feed and water *ad libitum*. The experimental period was 8 weeks. Data were analyzed using the PROC MIXED procedure of SAS (version 9.4, SAS Inst. Inc., Cary, NC, USA). Values of $P < 0.05$ were considered statistically significant. Dietary Cd exposure significantly reduced ($P < 0.05$) hen-day egg production, average egg weight, average daily feed intake (ADFI), feed conversion ratio, ovarian relative weight, and the counts of ovarian hierarchical (F5-F1) follicles compared to the CON.

Cd significantly increased reactive oxygen species (ROS) and malondialdehyde (MDA) in follicles and serum ($P < 0.05$) and significantly reduced the activity of superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPX), and the concentration of glutathione (GSH) in follicles and serum ($P < 0.05$). In addition, Cd significantly reduced the concentrations of estradiol (E2) and progesterone (P4) in the follicles and serum ($P < 0.05$). Meanwhile, autophagic vacuoles were observed in the Cd group using a transmission electron microscope (TEM). The Cd up-regulated the mRNA expression of autophagy-related genes *Beclin1*, *Dynein*, *ATG5*, *LC3-I*, *LC3-II*, and down-regulated the expression of *mTOR* ($P < 0.05$) in follicles. Moreover, the Cd up-regulated the mRNA expression of apoptosis-related genes *caspase-3*, *caspase-8*, *caspase-9*, and *Bax*, and down-regulated the expression of *Bcl-2* in follicles ($P < 0.05$). Se addition of 0.4 mg/kg showed significant ameliorative function in laying performance, oxidative stress, hormone, and the expression of autophagy and apoptosis-related genes ($P < 0.05$). In conclusion, our data suggested that dietary cadmium induces autophagy in follicular granule cells of laying hens via oxidative stress. Excessive autophagy was likely to cause apoptosis and lead to decreased egg production in laying hens, and selenium has an apparent antagonistic effect on cadmium.

Key Words: Heavy metal, Laying hens, Follicles, Oxidative stress, Autophagy

Microbiology and Food Safety

421P Assessing the *in vitro* effect of wild-type lytic bacteriophages on *Salmonella* Minnesota field strains.

Daiane Voss-Rech, Arlei Coldebella, Clarissa S. Vaz*; *Embrapa Suínos e Aves, Concordia, Santa Catarina, Brazil.*

Salmonella Minnesota has been commonly detected in Brazilian broiler's farms. Today, the interest in the use of lytic bacteriophages is growing as an alternative to conventional feed additives to reduce colonization of broilers by non-typhoidal *Salmonella* at pre-harvest. This study aimed to determine the *in vitro* effect of three wild-type lytic phages (BRM 13312, BRM 13313 and BRM 13314) against *S. Minnesota*. In total, 32 *S. Minnesota* field strains isolated from drag swabs from commercial broiler houses in southern and midwestern Brazil were analyzed. First, these strains were characterized by antimicrobial susceptibility test against 11 antimicrobials using the disk diffusion method and *Xba*I-pulsed-field gel electrophoresis (PFGE) analysis. Next, the ability of phages to lyse *S. Minnesota* strains was individually analyzed using the spot lysis assay in triplicate. Bacterial strains were cultured at 37 °C for 18 h to obtain exponentially grown cultures. After dispensing 200 µL of each strain in 6 mL of nutrient broth-MgSO₄ containing 0.65% agarose, suspensions were mixed thoroughly and distributed onto nutrient agar containing 10 mM MgSO₄ to obtain overlay cultures. Ten µL of each phage was dispensed over the solidified agarose layer to give a multiplicity of infection of 1,000 phage particles to each bacterial cell. *Salmonella* Typhimurium ATCC 14028 and SM buffer were used as positive and negative controls, respectively. Plates were incubated at 37 °C for 18-24 h. Fisher exact test was used to compare the lytic effect of each phage on *S. Minnesota* strains. Overall, *S. Minnesota* strains showed a high diversity as revealed by 28 *Xba*I-PFGE patterns. One strain (3.1%) was fully susceptible to the tested antimicrobials. The remaining isolates showed resistance to amoxicillin with clavulanic acid (AMC); ceftiofur (CEF), streptomycin (STR), tetracycline (TET), and trimethoprim with sulfamethoxazole (TS), distributed in the following patterns: TET (34.4%), TET-TS (18.7%), TET-STR (15.6%), TET-CEF (9.4%), TET-STR-TS (9.4%), TET-CEF-STR (6.2%), and TET-AMC-CEF (3.1%). There was a difference in the effect of phages, as BRM 13314 produced clear lytic plaques in 28 (87.5%) *S. Minnesota* strains ($p \leq 0.05$). All tested strains were insensitive to the other phages. Phage-based products containing different bacteriophages intend to overcome bacterial resistance and lyse a wide variety of strains. Therefore, a cocktail replacing phages BRM 13312 and BRM 13313 would have a broader efficacy against Brazilian *S. Minnesota* field strains.

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

422P Use of a bacteriophage in feed to
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control *Salmonella enteritidis* in broilers in a contaminated feed model.

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Controlling *Salmonella enteritidis* (S.E.) in chickens is a priority for broiler producers since S.E. is often one of the top five serovars causing human illness. There can be multiple sources of S.E. for a broiler flock from the parent hen, rodents, flies, beetles and even their feed. Lytic bacteriophages have been shown to be effective in killing bacteria such as *Clostridium* or *Salmonella* when administered to the birds by spray or oral gavage. However, because bacteriophage are heat labile, they have not normally been used to reduce S.E. by application in the feed. The CTC-Bio bacteriophage is heat stable and can be added to feed that is pelleted. This study had 3 treatment groups: T1-not treated, T2-1000 gm/metric ton bacteriophage, T3-1500 gm/metric ton bacteriophage in heat treated crumble feed. Three replicates of each treatment had 30 birds/replicate pen. The amount of feed for seven days (fed on days 8 to 14) was inoculated with meat and bone meal (MBM) contaminated at 10⁷ CFU/g S.E. in the inocula MBM for a 10⁴ CFU/g S.E. in the final feed. A chromium marker was also used to insure uniform distribution of the S.E. MBM. Cloaca swabs were taken from 10 birds per pen on days 14 and 21. On day 28 twenty-five birds ceca were cultured and ten birds spleen/liver were sampled. S.E. prevalence and enumeration were performed with tetrathionate, then XLT-4 (25 µg/ml nalidixic acid) with enumeration by micro most probable number (MPN) method of Berghaus, 2013. All statistical testing assumed a two-sided alternative hypothesis, $P < 0.05$ was considered significant. There was a numerical S.E. reduction in prevalence and number in the 14 and 21 day cloaca swabs. The estimated mean (SEM) S.E. log₁₀ MPN/cloaca swab based on a Tobit regression model were significantly reduced with 1000 gm/ton at -1.21^b, the 1500 gm/ton at -1.01^{ab} and the control at -0.32^a. There was no significant reduction in liver/spleen S.E. prevalence. There was a significant reduction in 28 day ceca S.E. prevalence with 1000 gm/ton at 53.3%^a, 1500 gm/ton at 57.3%^a, and control at 80.0%^b. *Salmonella* MPN in ceca based on Tobit censored regression had both treatments with significantly lower log₁₀ MPN/g than the control. This study demonstrated that in feed bacteriophage treatment can be an effective means of reducing S.E. colonization of broilers ceca from feed that has been contaminated with *Salmonella enteritidis*.

Key Words: *Salmonella enteritidis* in feed, bacteriophage

control

423P Effect of *Bacillus subtilis* and *Bacillus licheniformis* probiotic supplementation on performance and *Campylobacter jejuni* load in broilers challenged with *C. jejuni*. Revathi Shanmugasundaram*¹, Todd Applegate², Ramesh Selvaraj²; ¹Toxicology and Mycotoxins Research unit, US National Poultry Research Center, Athens, Georgia, United States, ²Department of Poultry science, University of Georgia, Athens, Georgia, United States.

Campylobacter jejuni is a food borne pathogen of poultry and controlling *C. jejuni* loads in poultry can be expected to decrease the poultry related *C. jejuni* outbreaks in humans. *Bacillus subtilis* and *Bacillus licheniformis* are potential probiotics in poultry production. The overall objective of this research was to determine the effects of *B. subtilis* and *B. licheniformis* probiotic supplementation on performance and cecal *C. jejuni* load in broilers challenged with *C. jejuni*. A total of 450 one-day-old broiler chicks were randomly distributed to five experimental groups. 1) Control; 2) *C. jejuni* infection; 3) 10 mg *B. subtilis* + 100 mg *B. licheniformis* /Kg feed + *C. jejuni* infection; 4) 5 mg *B. subtilis* + 50 mg *B. licheniformis* /Kg feed + *C. jejuni* infection; 5) 1 mg *B. subtilis*+10 mg *B. licheniformis* /Kg feed + *C. jejuni* infection. Each treatment was replicated in 6 pens with 15 chicks per pen. At 14 days of age, birds in treatment group 2-5 were challenged with 1×10^8 of *C. jejuni* via oral gavage. Chicken BW gain when fed diets supplemented with 10 mg *B. subtilis* + 100 mg *B. licheniformis* /Kg feed was not different than the unchallenged or challenged control birds at d 42 but had lower ($P < 0.05$) 0-21d and 0-28 d feed consumption and better feed-to-gain from d 0-21, 0-28, and 0-42 d than those in the unsupplemented *C. jejuni* infected group. Chickens challenged with *C. jejuni* had higher levels of cecal *C. jejuni* compared to the chickens in the control group. Chickens fed diets supplemented with 10 mg *B. subtilis* + 100 mg *B. licheniformis* /Kg feed and challenged with *C. jejuni* had approximately lower ($P < 0.05$) cecal *C. jejuni* loads than that in the challenged control group at 21, 28, 35, and 42 d of age. Chickens challenged with *C. jejuni* had higher levels of anti-*C. jejuni* specific IgA in the bile at all time points studied. There were no significant effects ($P > 0.05$) on serum anti-*C. jejuni* IgG amounts, jejunal villi height or crypt depth or villi height: crypt depth ratio between the birds in the treatment groups at any of the time points studied. It can be concluded that supplementing 10 mg *B. subtilis* + 100 mg *B. licheniformis* /Kg feed can improve production performances and decrease *C. jejuni* loads in poultry.

Key Words: Probiotics supplementation, *Bacillus*, *Campylobacter*, chickens, immunological response

424P Evaluation of the antimicrobial effect of essential oils associated with organic acids against serovars of *Salmonella* and *Escherichia coli* APEC. Beatriz

Pasqualli Fernandes*¹, Lilian Kolling Girardini², Amanda de Souza da Motta¹, Julcimar Machado Maciel², Márcio Rennan Santos Tavares³; ¹Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil, ²University of the West of Santa Catarina (UNOESC), Xanxerê, Santa Catarina, Brazil, ³Federal Institute of Sertão Pernambucano, Pernambuco, Brazil.

The use of growth promoting antibiotics to control pathogens and as performance enhancers is an established practice in the poultry industry. However, this use has been linked to the selection and development of bacterial strains resistant to antimicrobials, a fact that has unique health implications. Thus, alternatives are sought to the use of these while maintaining quality and productivity gains. In this context, the objective was to evaluate the antimicrobial potential of essential oils associated with organic acids already available on the market. Were evaluated by a method in silico (molecular docking) the potential of essential oils of oregano (*Thymus capitatus*), clove (*Eugenia caryophyllata*), rosemary (*Rosmarinus officinalis*) and propionic, lactic and formic acids to inhibit synthesizing protein peptidoglycan, D-glutamate ligase (PDB ID: 1E0D) and catalysts for DNA supercoiling, DNA gyrase B (PDB ID: 4PRV). As a control, the affinity of the antibiotic Ciprofloxacin against these was evaluated. The Checkerboard was also carried out to assess the synergy between formic acid and clove essential oil against isolates of *Salmonella Typhimurium* (ATCC 14028), *Salmonella Enteritidis* (ATCC 13076), *Salmonella Gallinarum* (Avian typhus) and *Escherichia coli* APEC (Cellulite). The results obtained in the in silico evaluation showed that among the six compounds evaluated, the lowest values of energy binding to the protein D-glutamate ligase were obtained for clove essential oil (-10.1697) and formic acid (-30.332), these energies, lower than those observed for Ciprofloxacin (12.0001). For DNA gyrase B, clove essential oil demonstrated greater affinity, with a binding energy of -94.4544, similar to that obtained for Ciprofloxacin (-98.6632). In the checkerboard, considering the compounds that obtained greater affinity in relation to the evaluated structures, the synergy between formic acid and clove essential oil was tested. A synergistic effect was observed against *Salmonella Typhimurium* (Σ FIC 0.37), *Salmonella Enteritidis* (Σ FIC 0.07) and *Escherichia coli* APEC (Σ FIC 0.265), demonstrating that the effect of the associated compounds is superior to the individual effect. For *Salmonella Gallinarum* (Σ FIC 0.515) the result of the association was additive, where the final effect is equal to the sum of the isolated effects, in which the concentration of the compounds necessary for the bactericidal effect was decreased. The results demonstrate that the tested products have antimicrobial properties, with potential to act in fundamental structures for the survival of microorganisms and the association of these allows bactericidal effect in reduced concentrations when compared to the use of the compounds alone.

Key Words: growth promoters, molecular docking,

checkerboard, essential oils, organic acids

425P Prediction of deoxynivalenol in wheat bran samples using Near Infrared Reflectance Spectroscopy (NIRS). Denize Tyska*^{1, 2}, Giséle P. da Rosa¹, Daniel F. Soares¹, Eduarda d. Gubiani¹, Rodrigo d. Carvalho¹, Raul F. Marcon¹, Adriano O. Mallmann², Carlos A. Mallmann¹; ¹Department of Preventive Veterinary Medicine, Federal University of Santa Maria, Santa Maria, Brazil, ²Pegasus Science, Santa Maria, Rio Grande do Sul, Brazil.

Mycotoxins are secondary metabolites derived from several filamentous fungi, mainly those belonging to *Aspergillus*, *Fusarium* and *Penicillium* genera. *Fusarium* species produce deoxynivalenol (DON), a toxic substance that causes damage when animals are fed contaminated diet. DON is often found in wheat and its by-products, such as wheat bran, which is commonly included in poultry diets. Therefore, this material must be monitored for the presence and levels of mycotoxins. The demand for fast and reliable methodologies for mycotoxicological analysis has made optical methods, such as Near Infrared reflectance spectroscopy (NIRS), a very promising tool. Thus, this study developed a tool for predicting DON in wheat bran samples using NIRS technology. Three hundred samples were split into two datasets: a calibration group, including 254 samples, and an external validation group, formed by 46 samples. The samples were received and promptly analyzed at the Laboratory of Mycotoxicological Analyses (LAMIC), Santa Maria (Brazil), by Liquid Chromatography Coupled to Tandem Mass Spectrometry (LC-MS/MS; reference method) in 2019 and 2020. Another fraction was used for optical data collection in order to build the spectra library. The spectra were obtained in a Foss XDS Rapid Content[®] Analyzer. The data were extracted and converted into a JCAMP file. The final spectral data were exported to conduct the chemometric analyses using the Unscrambler v.9.7 software (CAMO, Norway). DON levels varied from 200 to 3,140 $\mu\text{g}\cdot\text{kg}^{-1}$; mean value and standard deviation were 568 and 421.8 $\mu\text{g}\cdot\text{kg}^{-1}$, respectively. Partial least squares was the regression method applied in the models, using cross-validation. The calibration results for DON were: correlation coefficient, 0.94; coefficient of determination, 0.89; root mean square error of prediction, 178; and residual prediction deviation, 2.40. Values of the external validation dataset were compared with the levels obtained via LC-MS/MS and the Mann-Whitney test was applied; no statistical difference was found between the groups (p : 0.706), thus indicating a good ability to predict DON in wheat bran.

Key Words: Deoxynivalenol, wheat bran, prediction, chemometric analyses, NIRS technology

426P Phytochemicals as an alternative to conventional chemicals for controlling *Campylobacter jejuni* in poultry. Basanta Wagle*¹, Annie M. Donoghue², Palmy R. Jesudhasan²; ¹Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, ²Poultry Production

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Campylobacter, a leading cause of foodborne illness in humans, is associated with the consumption and/or handling of poultry and poultry products. Current strategies employed to reduce *Campylobacter* in live chickens provide inconsistent results indicating the need for an alternative approach. This study investigated the efficacy of phytochemicals namely, turmeric, curcumin, allyl sulfide, garlic, and ginger oil to reduce *Campylobacter* in poultry and delineated the underlying mechanism. A total of four experiments were conducted on the thigh skin of the chicken. Samples were inoculated with 50 μL ($\sim 10^6$ CFU/sample) of *Campylobacter jejuni* strain S-8 and allowed to adhere for 30 min. Skin samples were dipped into their respective pre-chilled treatment solutions (0, 0.25, 0.5%) at 4° C for an hour to represent chilling tank treatment followed by plating to enumerate *C. jejuni* ($n=3$ samples/treatment/trial). Both phytochemical-treated and untreated cells were stained with LIVE/Dead BacLight[™] bacterial viability stain and observed under the fluorescent microscope. The counts were logarithmically transformed, and data were analyzed by GraphPad version Prism 9. We found that the counts of *C. jejuni* were significantly reduced by 1.0-1.5 Log CFU/sample with garlic or ginger oil at a concentration of 0.25% and 0.5%. These compounds were as effective as 220 ppm of peracetic acid (industry controls). The viability test revealed that the cells treated with all phytochemical treatments had compromised cell membranes indicating this as a mechanism that phytochemicals use to damage/kill *C. jejuni*. Moreover, the selected phytochemicals (except allyl sulfide) reduced the adhesion of *C. jejuni* to chicken embryo ATCC CRL-1590 cells ($p<0.05$). In addition, the bioluminescence assay revealed that all the phytochemicals reduced quorum sensing of *C. jejuni* ($p<0.05$). These studies strongly support that the use of aforementioned phytochemicals in the post-harvest poultry would reduce *C. jejuni* in poultry meat and further reduce the incidence of human *C. jejuni* infection. Funded in part by USDA-NIFA-OREI-2017-51300-26815.

Key Words: *C. jejuni*, Phytochemicals, Chill tank, Antibacterial, Postharvest poultry

427P Accelerated shelf-life testing in frozen chicken wings. Bárbara Oliveira*, Angela Junges, Lucas Wolf, Liris Kindlein; Departamento de Medicina Veterinária, UFRGS, Capao da Canoa, Rio Grande do Sul, Brazil.

The shelf life and physicochemical properties of meat products depend on the tissue composition and on the morphology of skeletal muscle fibers. This study evaluated the effects of different freezing temperatures storage over time on attributes related to the texture of chicken wings (red muscle fibers). To estimate the shelf life of frozen chicken wings, the proposed method was to determine the shelf lives at four of the higher sub freezing storage temperatures. The analysis consisted of exposing the frozen

wings to thermal stress over a determined period, periodically evaluating its physical stability through pH value, water holding capacity (WHC), color and shear force tests. The experiment was completely randomized and 5 batches of frozen wings (20kg/each) were maintained at different storage temperatures (-22°C "control test", -18°C, -12°C, -06°C) for 12 weeks. Data were subjected to a one-way analysis of variance (ANOVA) and the significance of the difference between means at 5% was determined by Tukey's HSD test using SPSS software. Chicken wings in all studied freezing conditions over 12 weeks showed no alterations on water-holding capacity; shear force or pH value, thus probably maintaining meat moisture and consequently, tenderness and flavor. However, the color analysis showed statistical difference between freezing storage -18°C and -12°C in the luminosity angle (64,09 VS. 60,93, $p < 0.05$) and yellow intensity (16,27 VS. 18,71, $p < 0.05$), suggesting that this temperature variation may influence the quality of the product. The integrity of most physicochemical parameters related to the retention of intracellular water in the muscle tissue showed a stability of the chicken wings in the storage temperatures and proved the applicability of the methodological proposal in evaluating chicken red muscle fiber. In conclusion, the frozen chicken wings can be stored in -18°C without compromising their physicochemical quality.

Key Words: accelerated test, freezing temperatures, meat quality, shelf-life, skeletal muscle fibers

428P Leveraging a large scale GI microbiome dataset for necrotic enteritis outbreak prediction. Joshua P. Lefler*, Brian Dirks, Grant Gogul, James Gaffney, Andrew Izzo, Mallory Embree; *Native Microbials, Inc., San Diego, California, United States.*

Advances in next generation sequencing technology have allowed for a better understanding of broiler microbiome changes and their association with gastrointestinal (GI) diseases. These broiler GI microbiome shifts can be leveraged to gain valuable insights on bird performance, health, and advise preventative measures. Here, we present an analysis focusing on necrotic enteritis (NE) using a large-scale broiler database consisting of thousands of gut microbiome samples from across the country. Small intestinal content was sampled from over 2,000 birds, both healthy and unhealthy in the context of NE, across 10 sites in the United States between October 2018 and April 2021. Metadata was also collected pertaining to bird age, performance, and overall health. Small intestinal microbiota was characterized based on the V1-V3 region of the 16S rRNA gene. Taxonomic analysis of the microbiome revealed that a healthy broiler small intestine had high levels of *Lactobacillus* (73.50% +/- 27.30%) plus two other Lactic Acid Bacteria (LABs, 4.90% *Enterococcus* and 4.38% *Streptococcus*) as well as low levels of other Firmicutes (*Tepidimicrobium*, *Pseudobacteroides*, *Terrisporobacter*, *Clostridium_XIVb*, *Corynebacterium*, *Romboutsia*, *Escherichia*, all < 3.00%). Multivariate coordination

analysis (PCoA) revealed that sampling site had the strongest effect on intestinal microbiome composition ($P < 0.01$). While core microbiome composition was consistent at the genus level between sites, variations at the species level were observed. A random forest classifier built on 80% of the sample catalogue managed to classify samples as representing an unhealthy bird with >90% accuracy and 60-70% precision. Case studies leveraging producer data suggest that sampling the GI microbiome of birds at 14-21 days of age was predictive of future NE breakouts using this model. While still in an early phase, these findings demonstrate promise in leveraging the microbiome as an indicator of bird health, paving the way for future diagnostic tools to predict pathogen outbreaks and provide actionable insights.

Key Words: Microbiome, 16S Sequencing, Necrotic Enteritis, Machine Learning

429P Antimicrobial efficacy of a plant-derived compound, carvacrol, against *Salmonella* Enteritidis on organic chicken carcasses. Divek V. T. Nair¹, Shijinaraj Manjankattil*¹, Claire Peichel¹, Annie M. Donoghue², Kumar Venkitanarayanan³, Anup Kollanoor Johny¹; ¹*Department of Animal Science, University of Minnesota, Saint Paul, Minnesota, United States,* ²*Poultry Production and Product Safety Research, University of Arkansas, Fayetteville, Arkansas, United States,* ³*Department of Animal Science, University of Connecticut, Storrs, Connecticut, United States.*

Poultry carcasses contaminated with *Salmonella* are a significant contributor of foodborne outbreaks in the United States. With multiple concerns over the use of some synthetic antimicrobials in processing, plant-based antimicrobials have surfaced to be a potentially viable option for industry sustainability. We previously reported that carvacrol (CR), a plant-derived phenolic compound, resulted in the highest reduction of *Salmonella* Enteritidis (SE) on chicken wings at chilling conditions, among several other compounds tested. This study validated the antimicrobial effect of CR against SE attachment on organic chicken carcasses under chilling conditions. Organic chicken carcasses were inoculated with a cocktail of four SE serovars [higher inoculum study (10⁸ CFU/carcass) or lower inoculum study (10⁶ CFU/carcass)] and immersed in the treatment solutions for 30 min at 4°C. The treatments included in the higher inoculum study were negative control (NC; non-inoculated carcasses immersed in water without any treatments), positive control (PC; carcasses inoculated with SE and immersed in water without any treatments), chlorine (CL; 200ppm), peracetic acid (PAA; 200ppm) and carvacrol [CR; 1% (v/v)]. Similar treatments, except CL, were used in the lower inoculum study. After 30 min immersion in treatment solutions, the samples were stored at 4°C. Surviving SE on the carcasses were determined on days 0 and 3 for the higher inoculum study and days 0 and 7 for the lower inoculum study. At least 6 full carcasses were included in each treatment group per time point in both

studies and data were analyzed using one-way ANOVA. In the higher inoculum study, PAA and CR treatments resulted in 1.9 and 5.4 log₁₀CFU/ml reductions of SE, respectively, on the carcasses on day 0 compared to PC ($P < 0.05$). However, CL yielded no significant reduction of SE ($P > 0.05$). In contrast, on day 3, CR resulted in a log reduction of SE whereas PAA maintained 1.2 log₁₀CFU/ml SE reduction ($P < 0.05$). In the lower inoculum study, consistent SE reductions were obtained with CR and PAA on days 0 and 7. The PAA resulted in 4.5 and 4.9 log₁₀CFU/ml reductions of SE on meat on days 0 and 7, respectively ($P < 0.05$). Similarly, CR yielded 5.0 and 4.6 log₁₀CFU/ml reductions, respectively, on these days. Additionally, PAA and CR treatments resulted in 5.0 and 3.0 log₁₀CFU/ml reductions of SE in treatment water on higher inoculum and lower inoculum studies, respectively ($P < 0.05$). Results of the study indicated that PAA and CR treatments are effective in controlling SE contamination on organic chicken carcass [Funded through USDA-NIFA-OREI-2017-51300-26815].

Key Words: Salmonella Enteritidis, Carvacrol, Peracetic acid, Organic, Chilling

430P Comparison of microbial populations from day-of-hatch chick digestive tracts and chick papers. John B. Adkins*^{GS 1}, James Krehling¹, Kaicie S. Chasteen¹, Cesar Escobar¹, Luis R. Munoz², Aidan A. Talorico³, Kenneth Macklin¹; ¹Poultry Science, Auburn University, Auburn, Alabama, United States, ²Poultry Science, Auburn University, Auburn, Alabama, United States, ³Poultry Science, Auburn University, Auburn, Alabama, United States.

A preliminary study was conducted to compare microbial presence in day-of-hatch chick digestive tracts to that found on chick papers lining hatchery baskets. Fifteen baskets each containing 100 chicks were assigned as sample groups from which two chicks per basket were randomly selected. Following euthanasia, a portion of digestive tracts from crop to colon were aseptically removed from each chick and pooled by sample group. Samples were diluted in phosphate buffered saline (PBS) with ten-fold dilutions enriched in tetrathionate broth (TTB) at 38°C for 48 hours. Non-enriched dilutions were enumerated on plate count agar (PCA), MacConkey agar (Mac), m-Enterococcus agar (Ent), xylose-lysine-tergitol 4 agar (XLT4), and *Pseudomonas* agar (Psu). Chick papers corresponding to digestive tract samples were collected following chick removal. A 4 cm² section from the center of each paper was aseptically removed and diluted into 4 mL PBS. As with the digestive tract samples, enrichment in TTB was carried out for chick papers and each sample was enumerated on PCA, Mac, Ent, XLT4, Psu, and potato dextrose agar (PDA). PDA plates were incubated at 22°C for 72 hours, all other plates and enrichments were incubated for 24 hours at 38°C. Data were normalized through a log₁₀ transformation and analyzed using Welch's two sample t-test. Statistical significance was determined at a 95% confidence level.

Counts found on PCA, Mac, and Ent were significantly different ($P < 0.05$) showing more bacterial growth in digestive tracts than on chick papers. Colony counts for digestive tracts on PCA, Mac, and Ent averaged 3.33×10^7 , 3.69×10^6 , and 1.02×10^7 CFU/g respectively. Colony counts for chick papers on PCA, Mac, Ent and PDA averaged 1.68×10^6 , 8.95×10^4 , 9.01×10^5 , and 1.94×10^5 CFU/cm² respectively. No growth of *Pseudomonas* or *Salmonella* was detected from direct plating or plating TTB enriched samples on Psu or XLT4. These preliminary results provide insight for the expected levels of microbiological populations within day-of-hatch chick digestive tracts compared to corresponding chick papers. This baseline can aid in identifying possible points of microbial transmission through fecal shedding between chicks before placement within broiler houses.

Key Words: Fecal Shedding, Hatchery, Microbiological Enumeration, Pseudomonas, Salmonella

431P Identification of the intestinal microbes linked to the growth rate in broilers. Jing Liu*^{GS 1}, Qing Yang¹, Kelsy Robinson², Glenn Zhang¹; ¹Animal and Food Sciences, Oklahoma State University, Stillwater, Oklahoma, United States, ²Poultry Production and Product Safety Research Unit, USDA-Agricultural Research Service (ARS), Fayetteville, Arkansas, United States.

Intestinal microbiota is critically important for animal health and productivity. However, the influence of the intestinal microbiota on animal growth remains elusive. This current study was aimed at identifying the bacteria that are associated with the growth of broilers in a commercial production setting. Ten Cobb-500 broilers with extremely high body weight (BW), 10 chickens with medium BW, and 10 chickens with extremely low BW were randomly selected for each sex from a house of approximately 10,000 chickens on day 42. Cecal contents of 60 animals were subjected to DNA isolation and 16S rRNA gene sequencing. Our results showed that there were no significant differences in alpha-diversity of the microbiota among different groups, but differences were observed in beta-diversity based on unweighted UniFrac. LEfSe analysis identified a number of differentially enriched bacterial features among different groups. Spearman correlation analysis further revealed a number of bacteria to be significantly correlated with BW ($P < 0.05$). Many short-chain fatty acid (SCFA)-producing bacteria were positively associated with BW, but in a sex-specific manner. For example, an Anaerostipes member and a Faecalibacillus member were positively correlated with BW in males, while three Alistipes members and a Butyrivibrio member showed a positive correlation in females. In conclusion, our study identified a set of potential bacterial biomarkers for growth efficiency in broiler chickens. These gut bacteria could be targeted for manipulation to improve the growth rate and production efficiency in chickens.

Key Words: Intestinal Microbes, Body weight, Broiler chickens

432P Prevalence of virulence and antimicrobial resistance genes in suspected APEC isolates collected from different extra-intestinal tissue of layer hen. Fozol K. Ovi*^{GS}, Pratima Adhikari, Li Zhang, Aaron Kiess; *Department of Poultry Science, Mississippi State University, Starkville, Mississippi, United States.*

Avian pathogenic *Escherichia coli* (APEC) is an extra-intestinal *E. coli* that causes colibacillosis in chickens. Widespread use of antibiotics has been implemented in the poultry industry to improve growth performance and to minimize the economic impact of bacterial diseases, which has resulted in a high prevalence of antimicrobial resistance (AMR) genes among APEC isolates. With the recent trend toward antibiotic-free rearing system, alternative preventive strategies have been mandated against this disease. A better understanding of the genotypes of *E. coli* and its contribution to the localization of *E. coli* in different extra-intestinal tissues is crucial to formulate such effective alternative strategies. Therefore, this study was conducted to evaluate the prevalence of 5 virulent genes (*iroN*, *ompT*, *hlyF*, *iss*, and *iutA*) and 18 antimicrobial resistance genes (*blaTEM*, *tetB*, *silP*, *aac3V1*, *silE*, *terX*, *pcoD*, *aadA*, *qacEΔ*, *int11*, *sul1*, *merA*, *aph31A*, *terY3*, and *terD*) in suspected APEC isolates. A total of 30 *E. coli* isolates were collected from the extra-intestinal tissues (liver, heart, and spleen) of layer hens in peak production. The samples were incubated in lactose broth for 16 hours, followed by incubation in MacConkey agar for 12 hours to isolate *E. coli*. Then the bacterial DNA was extracted using ThermoFisher GeneJet DNA extraction kit according to the manufacturer's instruction. The prevalence of the *ybbw* gene was evaluated in the extracted DNA samples to confirm those isolates are indeed *E. coli*. A pentaplex PCR was conducted to evaluate the prevalence of 5 virulent genes in the samples. AMR genes were evaluated individually to determine their prevalence. One-way ANOVA was performed to determine the influence of tissue type (liver, heart and spleen) on the prevalence of virulence and AMR genes. The result showed virulence genes like, *iroN*, *ompT* and *hlyF* had 66.67% prevalence among the isolates whereas, *iss* and *iutA* had 53.33 and 60% prevalence, respectively. The samples showed a wide range of prevalence of AMR genes, where *qacEΔ* and *terD* genes had the highest prevalence (93.33%), followed by *aac3V1* and *terY3* gene (83.33%). Genes responsible for the resistance against silver (*SilP* and *SilE*) had the lowest prevalence (13.33 and 20%), implying most of those isolates could be sensitive to silver. Among other antimicrobials, tetracycline (*tetB* 23.33%) and beta-lactam (*blaTEM* 40%) could also be effective against these isolates. There was no influence of tissue type upon the prevalence of virulence and AMR genes ($P>0.05$). Based on these data, we conclude that the predilection site of extra-intestinal *E. coli* in layer hens is not affected by the five virulence-associated genes tested in this study.

Key Words: Antimicrobial resistance genes, APEC, Layer hen, Prevalence, Virulence gene

433P Effect of prebiotics and essential oil combination on *Salmonella Enteritidis in-vitro*. Ishab Poudel*^{GS 1}, Claudia Castañeda¹, Aaron Kiess³, Alamanda Calvert², Pratima Adhikari¹; ¹*Poultry Science, Mississippi State University, Mississippi State, Mississippi, United States*, ²*BioMatrix Intl, Princeton, Minnesota, United States*, ³*Poultry Science, Mississippi State University, Mississippi State, Mississippi, United States.*

With the ban on antibiotics as feed additives, several feed ingredients have been studied to lessen several foodborne pathogens, including *Salmonella enterica* subsp. *enterica* Enteritidis (SE). In the current study, we evaluated the efficacy of a combination product to control the growth of a Nalidixic acid resistant *Salmonella* Enteritidis (SE^{NAR}) in the presence of a commensal poultry gut bacteria *Lactobacillus reuteri* (LR) in an *in-vitro* experiment. The combination product consisted of chicory root prebiotic, yeast extract, lactic acid, and essential oils. The experiment was conducted as a completely randomized design with a 2×4 factorial arrangement of treatments with two levels of LR (yes or no) and four selected concentrations of combination product 0.00%, 0.05%, 0.1%, and 0.2% (w/v). Treatments were as follows: T1- control, SE^{NAR} + LR; T2 to T4- SE^{NAR} + LR + increasing concentration of product; T5 control- SE^{NAR} alone; T6 to T8- SE^{NAR} + increasing concentration of product. SE^{NAR} was cultured twice, once in Tryptic Soy Agar (TSA) at 37°C for 24h, and then in Xylose-Lysin-Tergitol 4 (XLT4) with 200 ppm of nalidixic acid at 37°C for 24h. LR was cultured in de Man, Rogosa, and Sharpe (MRS) agar anaerobically at 37°C for 48h. One single colony of SE^{NAR} and LR was picked from XLT4 and MRS agar and inoculated in Tryptic Soy Broth and MRS, respectively, at 37°C for 20h. 1 ml of the monoculture of SE^{NAR} and LR were added to 8 mL nutrient broth with or without the product and incubated at 37°C aerobically. Bacterial counts were recorded at 0, 2, 4, 6, and 8 h by the spread plate technique in XLT4 aerobically and MRS anaerobically, plates were then incubated at 37°C for 48 h. Data were log-transformed and subjected to ANOVA using the MIXED procedure of SAS (V9.4). Product concentration interacted with time ($P=0.0428$) with respect to SE^{NAR} concentration. Approximately a 0.5 log reduction in SE^{NAR} concentration was observed with 0.05% and 0.20 % (w/v) of the product at 4, 6, and 8h. Similarly, the inclusion of LR interacted with time ($P=0.0016$) where a log reduction of 0.3, 0.2, 0.5, and 0.3 of SE^{NAR} was observed at 2, 4, 6, and 8 h, respectively. There was a trend towards significance ($P=0.059$) in product concentration and inclusion of LR. The combination of LR and test product (0.05% to 0.20 %) reduced SE^{NAR} concentration more than either alone. Product concentration interacted with time ($P=0.014$) with respect to LR concentration. Approximately a 0.5 log increase in LR was observed after 8 h in 0.10% and 0.05% as compared to no product. Follow-up *in-vivo* experiments are needed to validate the efficacy of the combination product in poultry against SE.

Key Words: Prebiotic, Essential oils, Probiotic, Salmonella

434P Effect of pimenta essential oil against multidrug-resistant *Salmonella* Agona and *Salmonella* Saintpaul in non-processed turkey breast meat and ground turkey. Shijinaraj Manjankattil*^{GS 1}, Grace Dewi¹, Claire Peichel¹, Timothy Johnson², Sally L. Noll¹, Carol Cardona², Anup Kollanoor Johny¹; ¹*Animal Science, University of Minnesota, St. Paul, Minnesota, United States*, ²*Department of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, Minnesota, United States*.

Salmonella Agona (SA) and *Salmonella* Saintpaul (SP) are included among the emerging drug-resistant *Salmonella* in turkey production. Rapid solutions to control these emerging pathogens are needed. In this study, pimenta essential oil (PEO) was tested as a processing antibacterial against SA and SP in experiments representative of different stages of turkey processing. In the first experiment, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of PEO against SA and SP were determined, separately. Separate culture tubes containing 10 mL of tryptic soy broth were added with different concentrations of PEO (0%, 0.005%, 0.01%, 0.05%, 0.1%, 0.25% and 0.5%) and inoculated with ~4.5 log₁₀ CFU of SA or SP/mL. The tubes were incubated at 37°C for 24 h before dilution and plating. In the second experiment, the efficacy of PEO in chilling water (4°C) was tested against SA and SP attached on to fresh skinless turkey breast meat, independently and as a cocktail inoculated at 3 to 3.5 log₁₀CFU/g. Treatments were 0% PEO or PAA, 2% PEO, 2.5% PEO, 500 ppm (0.05%) peracetic acid (PAA), and combinations of 2% PEO and 0.05% PAA, and 2.5%

PEO and 0.05% PAA. After providing 20 min attachment time for *Salmonella*, meat samples were separately dipped in treatment water kept at 4°C for 30 min, followed by dilution and plating. In the third experiment, PEO was tested against a cocktail of SA and SP in ground turkey meat. Ground turkey meat samples were inoculated with mixture of SA and SP (~3 log₁₀ CFU/g) and added with PEO (0% PEO or PAA, 0.25% PEO, 0.5% PEO, 2% PEO, and 2.5% PEO). Samples were stored at 4°C and *Salmonella* recovery was done at 0, 1, 3, 5 and 7 days. Experiments followed a completely randomized design and repeated six times (n=6). Statistical analysis was done using PROC-MIXED procedure of SAS. The MIC and MBC of PEO against SA and SP were 0.01% and 0.25% respectively. In the dip experiment, all treatments resulted in a reduction of 3-3.5 log₁₀ CFU/mL *Salmonella* in chilling water compared to PC ($P \leq 0.05$) irrespective of independent or combined inoculations of SA and SP. Treatments, 2% PEO, 2.5% PEO, PAA (500ppm), 2% PEO + PAA (500ppm), and 2.5% PEO + PAA (500ppm) resulted in a reduction of 1.03, 1.09, 0.85, 1.02, and 1.45 log₁₀ CFU/g on turkey meat, respectively ($P \leq 0.05$). In ground turkey, PEO at or above 2% resulted in 2 log₁₀ CFU/g of the *Salmonella* cocktail by day 1. PEO at 2.5% resulted in enrichment negative samples by 1 min, indicative of rapid killing effect of the compound ($P \leq 0.05$). Results indicate that PEO could be a plant-based processing antibacterial against SA and SP in turkey processing (MDA RARF #81824).

Key Words: Salmonella Agona, Salmonella Saintpaul, Pimenta essential oil, Peracetic acid, Post-harvest intervention

Physiology and Reproduction

435P Seminal characteristics of Mexican Creole roosters in winter and spring. Rosalía Ordaz-Contreras*¹, Arturo Pro-Martínez¹, Juan M. Cuca-García¹, Said Cadena-Villegas², Josafhat Salinas-Ruiz³, Diego Zarate-Contreras¹, Belén López-Pérez¹, Irma Ordaz-Contreras¹, Fernando González-Cerón⁴; ¹Livestock Program, College of Postgraduates Campus Montecillo, Texcoco, Mexico, ²College of Postgraduates Campus Tabasco, Cardenas, Tabasco, Mexico, ³College of Postgraduates Campus Cordoba, Cordoba, Veracruz, Mexico, ⁴Animal Science Department, Chapingo Autonomous University, Texcoco, State of Mexico, Mexico.

The reproductive potential of roosters is determined by the quality of their semen, which is influenced by genetic and environmental factors. The objective of this study was to evaluate the seminal characteristics of Mexican Creole roosters (*Gallus gallus domesticus*), in winter (W) and spring (S) seasons. Sixteen 58 week-old roosters were used. Semen was collected every seven days for 22 weeks (Wk) (9 Wk in winter and 13 Wk in spring, using the dorso-abdominal massage technique. Birds were kept in individual cages (60 cm x 60 cm x 60 cm), inside a poultry house with natural ventilation, adjusted by side curtains. The lighting program was 16 h light during the evaluation period. The roosters were fed with a diet containing 17% CP and 2800 kcal of EM kg⁻¹. Feed intake was 120 g of feed bird⁻¹ day⁻¹ and water was provided *ad libitum*. The variables evaluated were: semen volume (SEVOL, mL), sperm concentration (SCE, x10⁹ sperm mL⁻¹) and mass motility (MOTM, from category (C) 0 to 5). The category C0: No swirl-nil or sporadic oscillation of individual sperm; C1: No swirl-generalized oscillation of individual sperm only; C2: Very slow distinct swirl; C3: Slow distinct swirl; C4: Moderately fast distinct swirl; C5: Fast distinct swirl. The variables: SEVOL and SCE were analyzed using the GLIMMIX procedure of SAS, with repeated measurements over time and a LSD mean test with a significance level $\alpha = 0.05$. A mixed generalized linear model was used for the MOTM variable, with an ordinal multinomial response. The results indicated that the SEVOL showed a difference ($p < 0.05$) between seasons (W: 0.27 mL vs. S: 0.33 mL), while in the SCE no difference was observed ($p > 0.05$) between seasons (W: 2.44 x10⁹ sperm mL⁻¹ vs. S: 2.87 x10⁹ sperm mL⁻¹). It was observed that MOTM with C5 had a higher probability of being observed in winter and spring (W: 32.32% vs. S: 44.74%). The category with the lowest percentage of probability of being observed was C1 (W: 4.66% vs. S: 2.80%). In conclusion, in spring, the Mexican Creole roosters showed better performance in terms of volume of semen, sperm concentration and mass motility.

Key Words: *Gallus gallus domesticus*, season, semen volume, sperm concentration, mass motility

436P Effect of heat stress on ileal microbiota in modern
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broilers and their ancestor Jungle Fowl. Nima Emami*¹, Monika Proszkowiec-Weglarz³, Lori Schreier³, Elizabeth S. Greene², Travis Tabler¹, Sara Orłowski¹, Joseph Hiltz¹, Nicholas Anthony¹, Sami Dridi¹; ¹Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, ²Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, ³USDA, Beltsville, Maryland, United States.

Heat stress (HS) has negative effects on poultry welfare, health and productivity resulting in economic losses. Broiler chickens are particularly susceptible to HS due to their high metabolic rate, rapid growth and lack of sweat glands. The commensal intestinal bacterial populations have important physiological role in the host and could ameliorate the negative effect of stress on the host. The aim of this study was to examine the changes in microbiota in two different broiler lines (Giant Jungle Fowl (JF), and Modern Random Bred (L2015) during HS. Day-old broiler chicks from each population were raised under thermoneutral (TN) conditions until d 28. On d 28 birds were subjected to TN (24°C) or chronic cyclic HS (8 h/d, 36°C) condition till d 56. On d 56, six birds per treatment (n=6) were euthanized, and luminal (L) and mucosal (M) bacterial populations were collected from ileum (IL) for DNA isolation using PowerSoil kit (Qiagen). Libraries were constructed using Nextera XT (Illumina) kits following Illumina protocol with V3-V4 16S rRNA primers and sequenced using 600 bp v3 kit on MiSeq (Illumina). DNA sequences were analyzed using QIIME2 platform and SILVA 132 database for alpha and beta diversity, and taxonomic composition, respectively. Differences in bacterial relative abundance were analyzed using LEfSe. In order to predict the function of microbiota, data analysis was performed through the PICRUST pipeline and illustrated with STAMP software. The effect of HS on taxonomic composition of IL was greater in JF compared to L2015. In contrast, the effect of temperature on microbial function was more pronounced in L2015 compared to JF. JF birds were characterized by higher ($P < 0.05$) abundance of *Bacteroides*, *Faecalibacterium* and *Phascolarctobacterium* and lower ($P < 0.05$) abundance of *Lactobacillus* genera in comparison to L2015. In IL-L, the abundance of many bacterial genera including *Faecalibacterium* and *Sellimonas* was higher ($P < 0.05$) in JF in comparison to L2015. Differences in bacterial function among lines were decreased as a result of HS. There were 64 and 25 pathways at KEGG level 3 with distinctive enrichment between L2015 and JF in TN and HS condition, respectively. These data indicate that the effect of HS on taxonomy and predicted functions of microbiota was genetic and tissue dependent with highest impact on modern broiler lines and IL-M.

Key Words: Broiler chicken, Heat Stress, Genetic Line, Microbiota

437P Comparison of seminal characteristics of breeding Creole roosters of Mexico. Giselle G. Maldonado-Martínez^{*1}, Juan M. Cuca-García¹, Arturo Pro-Martínez¹, Tlaacélel Tapia-Estrada², Diego Zárate-Contreras¹, Rosalía Ordaz-Contreras¹, Gerardo Aguilar-Villarreal¹, Miguel Á. Matus-Aragón¹, Kevin B. Flores-de la Torre¹, Fernando González-Cerón²; ¹Livestock Program, College of Postgraduates Campus Montecillo, Texcoco, State of Mexico, Mexico, ²Department of Animal Science, Chapingo Autonomous University, Texcoco, State of Mexico, Mexico.

In reproduction, natural breeding and artificial insemination are effective techniques and used in the propagation of animals for their conservation and genetics improvement, the seminal characteristics are important for the reproductive performance, this information is limited in Creole roosters (*Gallus gallus domesticus*) of Mexico. The objective of this study was to compare the seminal characteristics in Creole roosters from different groups of parents. A total of 140 Creole roosters of 88 weeks of age were evaluated, divided into groups of 7 birds of full siblings, each group was considered a treatment (T). A semen sample was collected for each bird by the dorso-abdominal massage technique for the evaluation of fresh semen. The birds were housed in individual cages (60 cm x 60 cm x 60 cm) within a poultry house with a light regime of 16 h and with natural ventilation adjusted by side curtains. The roosters were fed a 17% CP diet and 2800 kcal ME kg⁻¹. Feed consumption was 120 g per animal⁻¹ and water was provided *ad libitum*. The variables analyzed were: semen volume (SEVOL), sperm concentration (SCE), percentage of live sperm (PLS) and abnormal sperm (PAS). The data were analyzed by an ANOVA with the GLM procedure of SAS. In the SEVOL variable, T20 (0.200 mL) was only different (P<0.05) from T1 (0.54 mL), T9 (0.72 mL), T13 (0.55 mL), T14 (0.45 mL), T16 (0.47 mL), T17 (0.44 mL) and T18 (0.41 mL) respectively, other comparisons were not different. In the SCE no differences were observed (P>0.05) between treatments, registering concentration values between 2.18 x 10⁹ sperm mL⁻¹ and 2.91 x 10⁹ sperm mL⁻¹. With respect to PLS T8 (83%) was different (P<0.05) to T4 (97%) and T16 (92%). Differences were observed in PAS (P<0.05) in T4 (0.30%), T5 (0.14%), T8 (0.29%), T11 (1.0%) and T15 (0.42%). In conclusion, birds from different groups of parents show variation in the characteristics of semen volume, percentage of alive and abnormal sperm.

Key Words: *Gallus gallus domesticus*, sperm concentration, reproductive performance, semen characteristics, semen volume

438P The effects of Coenzyme Q10 on reproductive performance of Japanese quail under cadmium challenge. Hamid R. Rafieian^{*UG 1}, Mahdi Zhandi¹, Mostafa Sadeghi¹, Ali Reza Yousefi², Havva Marzban²; ¹Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran (the

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Coenzyme Q10, as a highly functional antioxidant, protects cells against oxidative stress. This study was aimed to determine the effects of Coenzyme Q10 on reproductive performance of Japanese quail under cadmium (Cd) challenge. A total of 216 six weeks old Japanese quails (*Coturnix coturnix japonica*) were randomly allocated into three experimental groups (n=24 birds per group) with three replicates each, during 8 weeks of experimental period (6-14 weeks of age). Treatments consist of 1) Negative control (NC): feeding basal diet; 2) Positive control (PC): feeding basal diet and cadmium (Cd) administration, and 3) CdQ10: feeding basal diet supplemented with 900 mg CoQ10 per kg diet and Cd administration. The Cd (10 mg /Kg BW) was subcutaneously administered at weeks 5 and 6 of experiment. To tissue sampling, one day after each Cd challenge two birds were euthanized from each replicate (n=6 per group). Birds mortality and egg production were recorded every day. Diameter and weight of big yellow follicles, carcass, oviduct, uterus, and stroma weight were measured. Magnum and isthmus were histopathologically evaluated. During weeks 7 and 8, hatchability and chick quality were recorded. Data were analyzed using SAS 9.4 with the GLM and GENMOD procedures. Multiple comparisons among groups were conducted by Tukey's test and significant differences were declared at P<0.05. Results showed Cd administration increased (P<0.01) mortality rate in PC group compared to the NC and CdQ10 (32% vs 0%, and 18%, respectively). The CoQ10 feeding had no effect on carcass weight, stroma weight, hatchability, and the three largest yellow follicles weights and their diameters compared with PC and NC (P>0.05). However, dietary supplementation of CoQ10 improved hatchling quality in CdQ10 compared to PC group (P<0.01), but there was no difference between CdQ10 and NC groups (P>0.05). Although egg production in NC group was higher than the CdQ10, quails in the CdQ10 group produced more eggs compared to the PC group (78.28%, 48.72%, and 38.29% respectively). The oviduct and uterus weight were increased by CoQ10 even more than PC and NC (P<0.01). The thickness and height of primary and secondary folds in the isthmus and magnum were increased by COQ10 compared to PC (P<0.01). In addition, the thickness of isthmus, magnum serosa, and isthmus tunica muscularis were increased in CdQ10 group compared to PC (P<0.01) and NC (P>0.05). It can be concluded that COQ10 has beneficial effects on some reproduction characteristics of quail under acute oxidative stresses induced by Cd.

Key Words: Coenzyme Q10, Cadmium, Histology, Quail, Reproduction

439P Variation in Pekin drake testosterone levels and pen level fertility in relation to access to females during

rearing. Lindsey J. Broadus*^{GS 1, 2}, Brian Lee³, Maja M. Makagon^{1, 2}; ¹*Department of Animal Science, University of California, Davis, Davis, California, United States*, ²*Animal Behavior Graduate Group, University of California, Davis, Davis, California, United States*, ³*Maple Leaf Farms, Inc., Leesburg, Indiana, United States*.

Pekin ducks (*Anas platyrhynchos*) housed in commercial breeder settings are often reared in same sex groups to facilitate separate diet provisioning. A few female ducklings (imprinting hens) may be integrated into the male groups as a strategy for increasing the reproductive success of the flock. However, the relationship between this management practice and the reproductive success of the ducks has not been confirmed. Thus, the objective of our study was to assess the effects of early social experiences (physical or nonphysical access to females of the same age) on the circulating testosterone levels of drakes and egg fertility. The study was conducted in a commercial barn subdivided into 16 pens. The pens were organized in groups of two, with a walkway separating each group from the next. Pekin ducklings (N=1440) were placed into alternating all-female and all-male groups (8 groups/sex; 150 females or 30 males/pen). At 12 days of age, three female ducklings were placed into each of four male pens. At approximately 20 weeks of age (woa), adjacent male and female pens were combined creating 8 mixed-sex pens of 180 ducks each. Circulating testosterone concentrations were analyzed from blood plasma samples collected from 10 focal drakes per pen at five time points. A baseline sample was taken before the ducks reached reproductive maturity (15 woa), and additional samples were taken at 22, 28, 34 and 45 woa. Pen-level fertility was determined based on candling of eggs collected from 33 to 46 woa. Testosterone data were analyzed using a Linear Mixed Model in R, with duck as a random effect in the nested model. A Mann-Whitney U test was used to analyze fertility data, averaged by pen. Circulating testosterone levels were not impacted by rearing treatment ($p = 0.232$), but did vary by age ($p < 0.001$). Testosterone concentrations were lowest at 15 woa, peaked as the ducks became reproductively mature (at 28 or 34 woa), and declined by 45 woa. Except at 15 woa, we observed pronounced individual variation in circulating testosterone levels (ex. 1.31 ng/ml - 16.83 ng/ml at 34 woa). Egg fertility was not impacted by rearing treatment ($p = 0.142$), and remained between 80-90% starting at 36 woa. The results indicate that housing male and female ducklings in adjacent pens, without physical contact, does not impact circulating testosterone in drakes and is sufficient for promoting good hatchability in this strain of ducks.

Key Words: testosterone, fertility, Pekin duck, management, physiology

440P Adapting methods for Golgi-Cox staining from rodents to chickens to evaluate dendritic morphology in the hippocampus. Allison N. Pullin*^{GS 1}, Jason W. Loxterkamp², Maja M. Makagon¹, Pamela J. Lein²; ¹*Center for Animal Welfare, Department of Animal Science,*

University of California, Davis, Davis, California, United States, ²*Molecular Biosciences, University of California, Davis, Davis, California, United States*.

There has been increased interest in understanding the development of spatial abilities of laying hens, as more hens are housed in spatially complex systems. Most studies of spatial abilities rely on cognitive and behavioral measures. Research on related neural mechanisms is limited, partially due to a lack of chicken-specific protocols to visualize architectural maturation of neurons. We aimed to address this gap by adapting a rodent Golgi-Cox staining (GC) protocol to evaluate dendritic morphology in the chicken brain. We focused on the hippocampus, a brain region associated with spatial abilities, learning, and memory. Our specific goal was to modify the rodent protocol with an estimated volume of the chicken brain for determining quantities of staining solutions and refine neuron selection criteria to consistently target hippocampal regions and neuron types. Dekalb white pullets (N=37) were euthanized at 16 weeks of age (WOA), and the right hemispheres of their brains were removed for GC. In an effort to observe a variety of brain development, the pullets reared in floor pens, single-tiered, or two-tiered aviaries. Brains were stained following the GC protocol for rodents, with modifications. An estimated hemispheric volume of 2 cm³ was used to adjust solution volumes. The success of this modification was confirmed under the microscope after brain samples were sliced and plated. Next, we set out to establish rules for consistent and accurate selection of neurons for analysis by identifying the numbers and types of neurons (pyramidal or multipolar) stained in the three regions of the chicken hippocampus. We captured images of the slices of hippocampus at 4X magnification, then fused the images together in ImageJ to create an overview of the hippocampus. This procedure was repeated for multiple tissue slices for each pullet. Fusing of images was successful for 31 pullets, representing a total of 245 slices of hippocampus tissue. Across these tissue slices, a total of 677 stained neurons were identified as potential candidates for imaging. Multipolar neurons constituted 90.3% of the stained neurons identified. Of the multipolar neurons, the majority were found in the dorsolateral region (62.4%), followed by the dorsomedial region (20.8%), and the ventral region (16.8%). Our modified GC protocol for chickens at 16 WOA specifies brain volume estimates needed for staining neurons and recommends selection of multipolar neurons in the dorsolateral region for study inclusion. The protocol can be used to evaluate how pullet housing systems impact neurodevelopment and to link neural mechanisms to differences in cognitive and behavioral measures of spatial abilities.

Key Words: dendritic branching, Golgi-Cox, laying hen, neurodevelopment, pullet rearing

441P The effect of feeding Omega-3 fatty acids on secondary sexual characteristics of different strains of layer breeder roosters. Anna Laszczuk*^{GS 1}, Rosemary

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Polyunsaturated fatty acids (PUFA) are long chain fatty acids, with omega-3 fatty acids (n-3 PUFAs) and omega-6 fatty acids (n-6 PUFA) being the most common variants. There have been an increasing number of studies that have demonstrated effects of an appropriate ratio of PUFA on the fertility and testosterone levels in various species, including roosters. Our objective was to investigate the effects of feeding an n-3 PUFA enriched diet on secondary sexual characteristics of layer breeder roosters. Layer breeders from ISA Brown (ISA) and Shaver White (SW) strains were allocated to 2 dietary treatments during the rearing and laying periods: a control and n-3 PUFA enriched diet (extrude full fat flaxseed and pulses mixture, LinPRO). The birds were housed in single sex pens during rearing. At 19 weeks of age (WOA), breeding pens were allocated with 3 males (balanced systematically for weight) and 25 hens (n=16 pens, 48 roosters in total, 4 female pens/ strain and diet combination). At 20, 22, 24, 26 and 31 WOA, the roosters were weighed, their comb heights and lengths measured using calipers and their combs were photographed. Comb lateral surface area, comb perimeter and comb colour were determined using ImageJ software (NIH, USA). A generalized linear mixed model (GLIMMIX, SAS) in a

factorial randomized completed blocking design with a repeated measure was used to assess the effects of age, diet, strain, and their interactions. There were effects of age on body weight (BW) (F= 41.33, P<.0001), comb length (F= 52.47, P<.0001) and perimeter (F= 31.04, P<.0001). There was also an effect of strain on BW (F= 526.16, P<.0001), comb length (F= 337.39, P<.0001), and perimeter (F= 491.67, P<.0001) but not diet (P>.1000). Older birds and ISA roosters were heavier and older birds and SW roosters had combs greater in length and perimeter. Comb surface area also increased with age (F=63.88, P<.0001). Additionally, there was a diet x strain interaction (F=7.71, P=.0060), with SW having larger comb surface areas than ISA roosters but n-3 PUFA fed SW roosters having smaller comb surface areas than the control SW roosters. Comb height was also affected by age (F= 28.21, P<.0001) and a diet x strain interaction (F=5.05, P=.0257), with ISA roosters having shorter combs than SW and n-3 fed SW roosters having shorter combs than the control SW roosters. Comb colour was affected by age (F=4.72, P=.0011) and strain (F= 5.12, P=.0246), with birds at 20 weeks of age and ISA roosters having redder combs than older birds and SW roosters. The results indicate a dietary treatment effect on comb size of roosters of the white strain only. Further analysis is on-going to determine any effects on fertility and sexual behaviour.

Key Words: Omega-3 fatty acids, Layer breeder roosters, Strain, Body weight, Secondary sexual characteristics

Processing and Products

442P Use of pressure with ambient or heated water to clean broiler carcasses on the slaughter side of the processing plant. Douglas E. Cosby*³, Mike McIntyre¹, Mike McIntyre¹, Osman Koyun², Kim Ingram³, Arthur Hinton³; ¹*Spray Systems Company, Glendale Heights, Illinois, United States*, ²*Department of Animal and Dairy Science, University of Georgia, Athens, Georgia, United States*, ³*Poultry Microbiological Safety and Processing Unit, U. S. National Poultry Research Center, Athens, Georgia*.

The slaughter side of the processing plants is an overlooked area for controlling bacterial loads on broiler carcasses. Reducing the bacterial and/or fecal load prior to the scald tank is hypothesized to reduce cross contamination of carcasses. Three sites (pre-scald, post-scald and post-pick) were selected due to the required space for a new cabinet and the available space in the processing line. The objective was to evaluate a low-volume, fluidic nozzle with ambient water at 150 psi (Experiment 1) or heated water at 250 psi (Experiment 2) to remove bacteria from pre-scald, post-scald or post-pick carcasses. Carcasses (n=15/replication; 3 replications/experiment) for each experiment were obtained from a local plant and transported to the laboratory. Carcasses were hung in standard shackles on a pilot processing line. Breast swabs (pre-moistened sponges) were collected before and after carcasses were washed. Sponge samples were assayed for total aerobic count (TAC), Enterobacteriaceae (ENT), *E. coli* (TEC) and *Campylobacter* (*Campy*) counts. Paired t-tests were conducted. In Experiment 1, significant differences (p<0.05) for TAC, ENT, TEC and *Campy* reduction (1.04, 0.9, 1.0 and 0.7 log₁₀ cfu/mL, respectively) were observed for post-scald carcasses. In Experiment 2, significant reductions for TAC (2.0 and 1.3 log₁₀ cfu/mL) were observed on pre- and post-scald carcasses and 0.8 log₁₀ cfu/mL for TEC on post-scald carcasses. The redesigned cabinet (incorporating a new design for the spray manifolds which allows more efficient washing of the carcasses) combined with increased water pressure and temperature has the potential to reduce the bacterial load entering the evisceration line allowing later intervention strategies to be more efficacious.

Key Words: Carcass, Enterobacteriaceae, Campylobacter, Wash

443P The effects of potassium ferrate (VI) on the meat quality of chicken thigh meat. Wendy Attuquayefio*, Skyler Lewis, Michael R. Barnas, James L. McNaughton; *AHPharma, Inc., Hebron, Maryland, United States*.

Potassium Ferrate (VI) (CAS#: 13718-66-6) is a potent oxidizing agent used primarily on water and wastewater treatment because of its antimicrobial and coagulating properties. The use of ferrate (VI) for disinfecting poultry

carcasses and wastewater during processing is being explored. However, there is concern that potassium ferrate (VI) may impact chicken meat quality, and studies on the effect of potassium ferrate (VI) on chicken meat quality are limited. The objective of this study was to determine the impact of different concentrations of potassium ferrate (VI) on the physicochemical quality of chicken thigh meat. Deionized water (positive control) and varying concentrations of potassium ferrate (VI) were sprayed onto approximately 100 g of boneless, skinless chicken thigh meat (N=3 per treatment), with unsprayed chicken thighs representing the negative control. A mixture of potassium ferrate (VI) and peracetic acid (PAA) was also tested (0.075% and 0.15%, respectively). The quality parameters assessed on the chicken thigh meat were color, pH, cooking loss, water-holding capacity (WHC), and lipid oxidation. Data were analyzed using the generalized linear model (GLM) procedure of SAS, and the treatment means were compared using Tukey's multiple comparisons test (P<0.05). Ferrate had no significant effect on color and WHC. The pH of the ferrate treatments was not significantly different; however, the 0.15% ferrate treatment had a significantly lower pH than the negative control group. The mixture of potassium ferrate (VI) and PAA significantly lowered cooking loss compared to the positive control group. In all, lipid oxidation was higher in cooked meat than raw meat, with the ferrate and PAA mixture treatment showing more lipid oxidation than other treatment groups. In conclusion, ferrate (VI) appears not to affect color and WHC but might impact pH, cooking loss, and the oxidative stability of chicken thigh meat. Further studies will be conducted to determine the effect of higher concentrations of potassium ferrate (VI) on meat quality and pathogen reduction on chicken breast meat.

Key Words: Ferrate, PAA, Sanitize, Disinfect, Wastewater

444P Study of the effect of using an acidity-regulating additive in water for scalding broiler carcasses. Lediane Tomazi Miotto*¹, Beatriz Pasqualli Fernandes¹, Caroline Schmidt Facchi¹, Diego Todescato¹, Liris Kindlein²; ¹*Research and Development, BTA Aditivos LTDA, Xanxerê, Santa Catarina, Brazil*, ²*Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil*.

The requirements imposed by domestic and foreign markets, related to the integrity of foods from animal origin, have contributed to implement quality control tools in the poultry industry in order to produce safe food. The present study focuses on the development of a technological innovation, which suggests the use of an acidity regulating additive in the water used in the scalding process of chicken carcasses. A total of 30 carcasses were used, divided into 5 treatments consisting of 6 samples each. The product, Aqualine®, was developed and supplied by the company BTA Aditivos LTDA, and was tested under the following

conditions: A - Control (no use of additive), pH 6.5 and 60°C; B - Aqualine®, pH 8 and 60°C; C - Aqualine®, pH 9 and 60°C; D - Aqualine®, pH 12 and 60°C and E - Aqualine®, pH 9 and 57°C. After applying the protocol, qualitative characteristics of the carcasses were measured: general appearance of the carcass skin, appearance of the breast muscle and ease of plucking, using a three (3) point hedonic scale. In addition, physicochemical parameters of scalding water were evaluated: total phosphorus (mg/L), pH, total alkalinity (mg/L) and anionic surfactants concentration (mg/L). The results were statistically analyzed using ANOVA and the means were compared amongst themselves using the Tukey test at 0.05% significance level. The sensory characteristics of the carcasses in the Aqualine® treated groups showed better appearance and ease of plucking when compared to the control group ($p < 0.05$). Anionic surfactants concentrations of the treated groups were also lower (< 0.2 mg/L) when compared to the control group (0.65 mg/L), showing a tendency for better indicators in the evaluation of physicochemical water parameters. The presence of anionic surfactants in the scalding water promotes the formation of foam, emulsification, and suspension of particles, demonstrating that the treatments do not generate these secondary reactions. For the phosphate parameter, no phosphate concentration increase was observed after the Aqualine® dosage. This is an important requirement since excess condensed phosphates and organically bound phosphates generate a buffering effect, thus hindering the action of alkalinizing agents. The results demonstrate that the water treatment with Aqualine® during the scalding process of chicken contributes to the improvement of plucking, carcass appearance, skin/breast integrity, as well as reducing the temperature of the scalding water, decreasing energy expenditure, and improving the quality of wastewater.

Key Words: Alkalinity, water quality, carcass quality, slaughterhouse, industrial process

445P Microwave analysis of broiler breast meat in conjunction with singularity value decomposition to categorize myopathic fillets. Aftab Siddique*^{GS 1}, Ryan freeman², Amit Morey³; ¹*Poultry Sciences, Auburn University, Auburn, Alabama, United States*, ²*Compass Technology Group, Alpharetta, Georgia, United States*, ³*Poultry Science, Auburn University, Auburn, Alabama, United States*.

Objective: Investigate the application of microwaves (2-18 GHz) as a rapid and a non-contact method to analyze woody breast myopathy in broiler breast fillets and using singular value decomposition to categorize the data. **Experimental design:** Radio-frequency spot probe antennas, launching and receiving microwaves over 2-18 GHz, were placed one above and one below the chicken breast fillet to record the frequency dependent reflected and transmitted power. A total of 80 breast fillets with varying levels of woody breast myopathy were scanned using the microwave (1601 frequencies per fillet in 20 sec.). The high dimensionality of the large dataset was decomposed using singular value decomposition and used for meat classification. **Material and methods:** Freshly deboned ($n=80$) chicken breast fillets were collected from a local commercial poultry processor and analyzed for woody breast (WB) by hand palpation as normal and woody breast, followed by visual inspection for white striping (WS). Microwave spot probes were used to measure the breast fillet samples, recording the transmitted and reflected power as a function of frequency, from 2 to 18 GHz. Samples were placed on an analytic plate in the middle of the transmitter and receiver probes mounted on the stand. High dimensionality collected data (amplitude and phase) was transformed into low-dimensionality using a singular value decomposition (SVD) analysis algorithm. A genetic algorithm (this technique, is an approach to problem-solving that uses a practical method in order to produce solutions based on the theory of natural selection) was used to find the frequency range within the 2-18 GHz measured, that optimizes classification accuracy. **Results:** Microwave data analyzed using SVD analysis for a single transmitter and receiver probe showed a 100% and 78% accuracy of normal breast and woody breast respectively (mild, moderate and severe). The woody breast detection accuracy increased to 90% when only severe woody breast was compared to normal breast meat. The method was able to detect white striping (mild, moderate and severe) with 86% accuracy. Data collected from dual probe receiver and transmitter showed an increase of 3% detection accuracy during SVD analysis. **Conclusion:** Microwaves in conjunction with big-data analytics such as singular value decomposition can be used as a non-contact, rapid method to detect broiler breast muscle myopathies.

Key Words: Microwaves, radio-frequency waves, woody breast, Singular value decomposition algorithms, the non-contact detection system

Symposium Abstracts

446S Utilizing big data in poultry smart farming: Opportunities and challenges. Rozita Dara*; *University of Guelph, Guelph, Ontario, Canada.*

Poultry farms are gradually becoming high-tech environments which utilize technologies such as sensors, smart poultry management systems, and artificial intelligence to increase production, improve bird welfare, and minimize environmental footprint. These technologies generate large amounts of data by collecting a variety of parameters from farm operations. The data collected by these tools can offer credible insight into improving farm management, enhancing precision feeding, and early detection of infectious disease. However, governance, protection, and analysis of this large volume of data impose many challenges. This talk will discuss the impact of emerging digital technologies in poultry farming and opportunities they provide in terms of enhancing production and farm management. It will also review technology and operational requirements that enhance adoption and trust in farm technologies and also address challenges such as interoperability, data security, and sustainability.

Key Words: Big Data technologies, data collection and analysis, data sharing and privacy, data platforms

447S Identifying novel strategies to improve animal health and wellness: From concept to product. Jordan M. Sand*; *Ab E Discovery, Middleton, Wisconsin, United States.*

Animal agriculture, under public pressure, has moved to raise animals without use of growth promoting antibiotics, in part due to development of antibiotic resistance beyond the farm as well as perceived benefits of raising an animal under more natural conditions. Because of these perceptions, many products sold as antibiotic replacements have flooded the market that may improve animal efficiency and health. To move the needle in identifying economic, innovative products that improve animal production which do not fall under an antibiotic or drug category requires a novel approach. Using a series of statements to guide our thinking for finding novel products, our team led by Mark Cook identified that one area of high growth potential was in animal co-products, i.e., discarded organs and tissues, harvested during animal slaughter. While ~70% of the animal carcass is used for consumption, ~30% is rendered for low-value products. These co-products are largely derived from tissues and organs that serve key biological functions, including brain, spleen, and gastrointestinal tract tissues. From this low-value stream of animal co-products, we developed a number of high-value therapeutics and antibiotic replacements that address key issues in companion and production animal health, including Cosatein (Cook Sand Protein) derived from porcine mucosa and Cosajaba (Cook Sand Jake Barry) oil derived from the preen gland of chickens and turkeys. Cosatein results in

improved weight gain and feed efficiency in chickens and improved dermatitis in mice and dogs. Cosajaba oil contains odd chain fatty acids that when incorporated into the diet, reduces swelling and overall joint inflammation in murine arthritis models. Additionally, Cosajaba oil incorporation into in non-domesticated fish diets reduced mortality and cortisol due to handling and environmental stressors. Additional, high-value products with biological properties have yet to be discovered. The increased focus on a potentially rich stream of bio-active products from the rendering stream may provide an avenue for antibiotic replacements that promote animal health and wellness while improving the sustainability and profitability of raising livestock and poultry.

Key Words: Antibiotic Alternatives, Growth Promotion, Animal Byproducts, Animal Coproducts

448S Applying novel molecular biology approaches to identify new targets that promote avian health. Elizabeth A. Bobeck*; *Animal Science, Iowa State University, Ames, Iowa, United States.*

As the poultry industry works towards optimizing new housing systems, moving towards antibiotic-free production, maintaining sustainability, and responding to other consumer pressures, it is paramount that the scientific community identifies molecular mechanisms and uses these discoveries to develop novel ways to improve poultry health and production. Using new molecular technology or repurposing existing technology to improve poultry health and production reflects a major frame shift in poultry research. While understanding the impact of feedstuffs, additives, nutrition, genetics, management, environment and performance on poultry performance, namely FCR, has historically been the gold standard, it is now apparent that applying molecular work to traditional performance studies is the new normal in poultry research. Recent technology including RNAscope, a molecular method that combines PCR and immunohistochemistry, will allow researchers to simultaneously detect protein with localized mRNA expression analysis. Other techniques such as Seahorse metabolic assay allow further inquiry into cellular preferences under baseline and stressed conditions in a cellular monolayer. The metabolic assay allows for stressors to be applied at both a cell and whole animal level. As reagents for cellular analysis improve, flow cytometry continues to play a key role in immune and reproductive work, while microbiome and metatranscriptome sequencing pushes our understanding of gut ecology and related fields forward. A second key for success is continued research collaboration between fields to integrate findings and maximize outcomes. Using new technologies and managing collaborative projects across scientific fields will lead to novel byproducts, feedstuffs, additives, and other dietary components that will find success in the poultry industry.

Key Words: flow cytometry, immunometabolism, microbiome, RNAscope, seahorse metabolic assay

449S Tapping into the immunological potential of the avian species: Novel strategies to improve performance.

Vanessa Leone*; *Department of Animal & Dairy Sciences, University of Wisconsin, Madison, Wisconsin, United States.*

A key component of maintaining poultry health and welfare to maximize sustainability and production outcomes is understanding and leveraging the avian immune system. Diminished flock health is often a trade-off for genetic selection of high production efficiency traits in birds. To overcome immune fragility, bird health must be carefully managed through various measures, including vaccinations, biosecurity, and husbandry practices. With consumer pressure for decreased use of antibiotics across the poultry industry, there is dire need for safe and effective alternatives that exhibit health-promoting properties. Dietary manipulations through the use of feed additives offer affordable, easy-to-administer alternatives. In the mid 1990's, Mark Cook, Michael Pariza, and colleagues identified and isolated microbially- and host-derived positional and geometrical isomers of the fatty acid linoleic acid (C18:2n-6) in cooked, ground hamburger meat. Termed Conjugated Linoleic Acids (CLA), inclusion of A 50:50 mixture of man-made derivatives of the predominant isomers, cis-9, trans-11 (c9,t11) and trans-10, cis-12 (t10,c12) in animal feed, including poultry diets, has shown remarkable capacity as an anti-inflammatory fat. In birds, CLA can prevent downstream inflammatory cascades in the face of immune challenge with mixed impact on FCR and growth rate. Further, given human health implications for CLA and its high incorporation rates into animal tissues meant for human consumption, CLA's dietary inclusion into broiler diets, for instance, may offer unique functional food opportunities for the poultry industry. CLA serves as one example of a compound that can reduce inflammation in poultry with unique potential benefits from a farm-to-fork perspective. However, additional novel compounds are needed in order to manage the avian immune system across the poultry industry. Feed additives and strategies that focus on host- microbe interactions to maintain gut health and mucosal immunity are of key interest. Given CLA's initial discovery as a microbially-derived lipid with anti-inflammatory properties as well as advances in microbiome sciences, there are unique opportunities to mine the gut microbiota of poultry and livestock to identify compounds that can enhance bird mucosal immunity. These compounds can then be applied directly into poultry diets as postbiotic feed additives. By combining basic and applied poultry, immunology, and microbiology approaches, advances can be made in the management of bird health.

Key Words: Conjugated linoleic acid, anti-inflammatory, microbiome, antibiotic alternatives

450S Searching the microbiome for druggable targets:

Poult. Sci. 100 (E-Suppl 1)

Lessons from the avian gut. Steven C. Ricke*; *Meat Science and Animal Biologics Discovery, Department of Animal and Dairy Sciences, University of Wisconsin, Madison, Wisconsin, United States.*

The avian gastrointestinal microbial population in the mature bird consists of a highly diversified and complex microbial ecosystem. A wide range of microorganisms inhabit the avian GIT with the microbial populations differing in the crop, small intestine, and ceca, respectively. In addition to age of the bird, the environment it inhabits can also be an influential factor with differences being detected between fully housed birds versus those raised in freerange settings. The metabolic activities in the microbial populations in each segment of the avian GIT can also vary as a function of nutritional substrate availability and the level of oxygen present in the lumen versus the intestinal wall. As the GIT environment becomes more anaerobic, strict anaerobic organisms such as methanogens become established as part of the microbial community. In addition, there are several groups of GIT microorganisms which can hydrolyze and ferment different dietary carbohydrates, lipids, and proteins. The resulting fermentation products can range from short chain volatile fatty acids and lactate to ammonia, hydrogen, carbon dioxide, and methane. In depth metabolomics on cecal contents has revealed several hundred metabolites many of which remain unidentified. Historically, the avian GIT has been a source of probiotic candidates that can be introduced as direct fed microbials to limit pathogen GIT colonization and more generally promote a healthy GIT that is beneficial to the bird. Given the numerous metabolites detected in the avian GIT, it is quite possible that the avian GIT could also serve as a source of compounds with a multitude of beneficial functions. This presentation will discuss the potential for harvesting beneficial microorganisms and their metabolites and screening methods for assessing their functional properties.

Key Words: Beneficial bacteria, metabolites, gastrointestinal tract, fermentation, foodborne pathogens

451S Catastrophic circumstances and commercial flock euthanasia: Meat type poultry.

Gregory P. Martin*; *Cooperative Extension, Penn State University, Lancaster, Pennsylvania, United States.*

Meat type poultry raised on litter floor based housing pose a challenge during a catastrophic event that requires quick action to humanely euthanize an entire flock while maintaining high biosecurity on site. Several factors will determine the best method of euthanasia used. If only a few birds remain alive during an event, individual methods of euthanasia could be employed. For larger flocks, mass depopulation methods including fire fighting foam, mixed gas injection, and (as a last resort) ventilation shut down with heating could be employed to render a quick end with the lowest amount of workforce exposed. All commercial poultry farms should have a 3D (depopulation, decontamination, disposal) plan to anticipate what resources would be needed for efficient process, keeping in mind

building configuration and flock size. Training on key apparatus, confirmation of death, and proper handling of deadstock are key to effective and safe outcomes of handling a catastrophic event.

Key Words: euthanasia, foam, emergency, safety, chicken

452S Catastrophic circumstances and commercial flock euthanasia: Laying hen. Kenneth E. Anderson*; *Poultry Science, North Carolina State University, Raleigh, North Carolina, United States.*

Significant differences in management practices, bird type, and housing construction makes it difficult to prescribe the use of a single depopulation method across all farms in the event of a foreign animal disease outbreak. Timely and humane depopulation was identified as a critical measure to contain the outbreak. In Layer operations depopulation methods of CO₂ kill carts, CO₂ injection, and fire-fighting foam can be overwhelmed or not work prolonging suffering of infected birds. What are the humane aspects and effectiveness of ventilation shut down plus (VSD+) for depopulating caged laying hens? By evaluating EEG, environmental parameters, behavior, and stress physiology using 3 treatments VSD, VSD+Heat (VSDH), and VSD+CO₂ (VSDCO) in chambers. Followed by scale up tests of the systems in a room with stair-step cages. Data was analyzed with GLM with full factorial effects for CO₂ and heat were fit to each of several response variables and all pairwise comparisons used Tukey's HSD. The transformed EEG analysis used the integral area under the curve calculated using the Trapezoid method; using a NPARM analysis. The chamber temperature, % CO₂, and % RH similar at the start averaging 89 F, 0.22 % and 31.6 %, respectively. The VSDH temperature was higher at 107 F (P<0.0001) and VSDCO₂ %, respectively. RH increased (P<0.0004) in the VSD and VSDH to 62.5 and 66.0 %, respectively. The hen core body temperatures (CBT) ranged from 105.1 to 106.4 F. At time of death (TOD) the CBT was highest (P<0.0001) for VSD (113.1 F) and VSDH (115.3 F). The time to reach TOD was longest in the VSD at 1.53 hrs followed by VSDH, VSDCO, and VSDHCO the lowest at 0.15 hrs. Hen in the VSD treatment spent 82% of their time in an insensible (0-0.01mV) range while the VSDH, and VSDCO were at 56, and 65%, respectively. In the scale up the TOD increased for all methods and was very similar for VSDH and VSDCO.

Key Words: laying hen, cages, depopulation, ventilation shutdown

453S Use of vegetative buffers to mitigate poultry dust and ammonia. Paul H. Patterson*; *Penn State University, University Park, Pennsylvania, United States.*

The Clean Air Act (1970), CERCLA (1980) or Superfund and EPCRA (1986) have historically been one means to scrutinize the emissions of poultry CAFO's. In the wake of new emissions factors (AP-42's) coming for poultry from the EPA in 2022, best management practices to mitigate

both ammonia and particulate matter (PM) emissions will be helpful to navigate whole farm emissions. Vegetative filters are one conservation practice to help reduce poultry dust and ammonia emissions. The NRC Committee on Air Emissions from Animal Feeding Operations (2003) categorized ammonia emissions as having a major global, national and regional impact for atmospheric deposition and haze, but only a minor local affect. Emissions are a concern for land and water surface deposition. While PM and odor are major issues for local receptors impacting human health, atmospheric haze, and quality of life, when dispersed into a larger air-shed, dust and odor were viewed as insignificant on a global, national and regional scale. Poultry farm vegetative buffers come in many types and sizes but are predominantly used for six conservation practices: 1. To landscape/screen the farm and buildings from the road and neighbors, 2. Riparian buffers for water quality 3. Biomass buffers for bedding or fuel, 4. Energy conservation with a wind break, shade bank or snow fence buffer, 5. Shelterbelts cover for outdoor access birds, and 6. Vegetative filters for air quality (dust, ammonia, odor, etc.). Plant species vary in their ability to tolerate poultry house dust and ammonia, and their ability to capture these emissions. Generally, warm season grasses, shrubs and deciduous trees tolerate dust better than evergreens like Arborvitae and others. Vegetation type and architecture also affects their ability to trap dust e.g. simple vs. compound leaves, leaves vs. evergreen needles, and smooth vs. hairy and sticky leaves. Similarly, warm season grasses and legumes, shrubs and deciduous trees tolerate ammonia better than evergreens, especially the genus pinus. Leguminous shrubs and trees that fix nitrogen are especially tolerant of ammonia emissions. Strategically designed, vegetative filters can trap and treat poultry house dust and ammonia reducing downwind emissions. This presentation will review the vegetative filter design process on poultry farms for dust and ammonia emissions, discuss alternative plant species, soil analysis, site preparation, and the importance of maintenance (weed & pest control, irrigation etc.) to ensure proper establishment. Finally financial support, cost share programs and resources for information and plantmaterials will be provided.

Key Words: poultry emissions, vegetative buffers, dust, particulate matter, ammonia

454S How to communicate with poultry producers concerning disease recognition and control without farm visits. Fred Dustan Clark*; *Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States.*

The Covid-19 Pandemic created new challenges for Extension personnel "face to face" educational outreach efforts in that farms visits, in person seminars, short courses, meetings and other "normal" Extension activities were either restricted or limited. This is still at least a part of the current situation, although many limitations and restrictions have been lifted or modified. How did Extension faculty respond to these challenges? How can we as Extension

faculty respond to these outreach challenges? We did and will continue to respond in typical Extension fashion; we will adapt, modify, create, “think outside of the box”, and view these challenges as opportunities to develop something new. Many of the responses to the new educational outreach opportunities may become a part of the new Extension “normal”. Various methods of communicating to producers (commercial and small flock) about diseases and disease control without actually visiting the farm can be used. Some of these methods are types we normally use to at least begin our planning for a farm visit; such as a phone call, text message, or email. These methods can be intensified to gather information that we would normally gather on the farm ourselves by observations and questioning. Use of various current available electronic video communication platforms to communicate with our clientele are being used successfully. The use of telemedicine by means of telecommunications technology for diagnosis, recognition, and suggestions for treatment and/or control of disease is a concept utilized with increasing frequency in human medicine due to the risks from the pandemic; this same telemedicine concept is being used in veterinary medicine. Other creative ways of communicating with poultry producers can also be of tremendous value. Cameras that are now extremely small and of tremendous picture quality are owned by almost all, since phones have cameras present. The old proverb that a picture is worth a thousand words allows novel ways to visit farms to conduct our programs. In this presentation various successful methodologies for educational outreach will be presented. Cooperative Extension faculty are trusted by their clientele and expectations were and remain high while in this pandemic for us to provide accurate, research based information to producers in our states even when we cannot visit their individual farm for face to face educational outreach

Key Words: Outreach, Biosecurity, Diseases, Farm Visits, Communicate

455S Answering small flock poultry producer’s questions about lead toxicity and how to communicate with poultry interested clientele throughout the world. Jacqueline P. Jacob*; *Animal and Food Sciences, University of Kentucky, Lexington, Kentucky.*

Human exposure to heavy metals, especially lead, can occur through a variety of different pathways. These include the air, drinking water, food, contaminated soil, deteriorating paint, and dust. Many sources of heavy metals have been eliminated in the United States. For example, the manufacturer of lead-based paint was banned in 1978 and in 1996 the EPA took final steps to phase out leaded gasoline. However, the historical environmental legacy from these sources of heavy metals remains and can pose a health risk. Research has shown that heavy metals, especially lead, can be transferred from contaminated soil to eggs. With the dramatic increase in backyard egg production, concern about lead toxicity has grown. How can these concerns be addressed? During the COVID-19 many extension

personnel have moved what were local workshops online to zoom webinars. This has opened the presentations to people from around the world. There is considerable demand for educational programming from people in developing countries. Is this a need we as educational professionals can fill?

Key Words: Webinars, Lead in eggs, Backyard flocks, International outreach

456S Informal Nutrition Symposium: Leveraging the microbiome (and the metabolome!) for poultry production. Rosalina Angel*¹, Mamduh Sifri², Todd Applegate³, Doug Korver⁴; ¹*Animal and Avian Sciences, University of Maryland, College Park, Maryland, United States*, ²*Sifri Solutions, LLC, Quincy, Illinois, United States*, ³*Poultry Science, University of Georgia, Athens, Georgia, United States*, ⁴*Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, Alberta, Canada.*

Knowledge of gut microbiology of poultry has advanced from an ability to culture relatively few microbial species, to attempting to understand the complex interactions between the bird and its microbiome. This symposium will help poultry scientists to make sense of the implications of the vast amounts of information being generated by researchers.

Key Words: microbiome, nutrition, immunometabolism, kinomics, microbial endocrinology

457S Historical overview of the development of poultry microbiome research. Margie D. Lee*; *Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, Virginia, United States.*

Poultry scientists and poultry veterinarians were early adopters of microbiome biology. In the earliest reports, it is unclear whether feeding birds fermented products was applied for gut health or because this was a good way to dispose of refuse. The second application of microbiome biology, in the 20th century, was culture-based in that as cultivation methodology developed, the poultry normal flora was of great interest for its diversity of anaerobes. In addition, understanding the bacterial community of poultry was sought in order to manage intestinal pathogens, food safety, improve feed conversion and detect antimicrobial resistance. In this 21st century renaissance of the microbiome, molecular characterization of complex microbial ecosystems have enabled investigations into ecological diversity and its effects on intestinal health. These methodologies reveal the microbiomes’ effect on intestinal metabolome, glycome, and proteome leading to greater understanding of gut development, immune and disease status and production efficiency.

Key Words: microbiome, historical

458S Immunometabolism and metabolic health: Implications for poultry production. Ignacio R.

Ipharraguerre*; *Institute of Human Nutrition and Food Science, University of Kiel, Kiel, Germany.*

Immunometabolism is the reciprocal interaction between immunity and metabolism, which includes immune-related metabolic alterations and the metabolic programming of the immune response. From an evolutionary viewpoint, long-lived or low-grade inflammation incapable of pathogen elimination entails no advantages for the survival of the host. However, its occurrence may compromise tissues and associated functions critical for metabolic homeostasis. Within this framework, dietary interventions can affect immunometabolic processes by dictating the fitness of the system composed by the host and its gut microbiota. During the production cycle, numerous situations (e.g., nutritional imbalances, stress, infections) can generate failures in the functional integrity of the intestine. Frequently, these dysfunctions lead to the loss of mutualism between the microbiota and the host (dysbiosis), which dysregulate the microbial synthesis of biologically active molecules, enhance the entry of harmful agents into the epithelium and systemic circulation, and trigger the activation of an inflammatory response. If not resolved quickly and effectively, this pathophysiology can contribute to the development of diseases (enteric or metabolic) or immunometabolic inefficiencies that compromise the productive potential and health of food-producing animals, including poultry.

Key Words: Inflammation, Metabolism, Immunity, Dysbiosis, Bile acids

459S Kinomics: Regulation of the metabolome. Ryan Arsenaault*; *Animal and Food Sciences, University of Delaware, Newark, Delaware, United States.*

Kinomics is the study of kinases and their targets. Kinases are enzymes that post-translationally phosphorylate proteins. Unlike genomics or transcriptomics, kinomics involves modifications that directly alter protein function affecting cell or tissue phenotype. Phosphorylation is the most common post-translational modification and nearly every function carried out by the cell is regulated to some degree by phosphorylation. Indeed, many metabolic pathways and processes are regulated by kinase activity and phosphorylation. By studying kinase activity within these metabolic pathways, we can determine metabolic flux changes as well as the changes elicited by metabolites that serve as signaling molecules. Since the microbiome is a major source of metabolites, kinomics can open a window to understand how the microbiome, and changes in the microbiome, influence the host and inform intervention strategies.

Key Words: kinome, kinase, gut, signaling, immunometabolism

460S Microbial Endocrinology: How evolved intersections of microbiology and neurobiology matter to poultry health and nutrition. Mark Lyte*; *Dept. of*

Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, Iowa, United States.

The union of microbiology and neurobiology has led to a revolution in the way we view the microbiome. The poultry intestinal tract and the gut microbiome possess many of the same genes for producing and recognizing the exact same neurochemicals such as those involved in the stress response. This evolutionary shared neurochemistry represents a language with which the host can communicate with the microbiota in a bi-directional manner. Further, such shared neurochemistry with microbes is not limited to animals as the plants that are used for feed for poultry also contain some of the very same neurochemicals. Thus, all three elements, host, microbe and nutrition, interact. What this means for poultry health and the design of the next generation of feeds will be discussed.

Key Words: Microbiome, neurochemicals, gut, infection, nutrition

461S Applying the knowledge: Where is the research leading us? Brooke Humphrey*; *Phibro Animal Health, Maple Grove, Minnesota, United States.*

As we continue to learn about the microbial communities and their activities within the gastrointestinal tract it is becoming more clear how deeply integrated they are into host physiology. This requires a new look at what are often considered old problems as we integrate the microbiome into new problem solving approaches and innovations. To develop consistent and robust microbial solutions for poultry production, gaining a deeper understanding of microbiome function vs presence, the significance of microbiome integration into inter-organ metabolism and how microbiome specifically regulates innate and adaptive responses at mucosal surfaces are important knowledge gaps to be filled in order to fuel actionable insights. Given the broader impact of the microbial ecosystem on the host, a more collaborative and systems thinking approach are anticipated to yield the most fruitful learnings.

Key Words: microbiome, health, immunology

462S Strategic modulation of intestinal microbiome: A microbiologist's perspective. Robert J. Moore*; *School of Science, RMIT University, Bundoora, Victoria, Australia.*

The gut microbiome of chickens influences their health and productivity. Modulation of the taxonomic structure and functional capabilities of the gut microbiota can therefore be used to enhance health and productivity. Understanding what sort of changes in microbiota are desirable and how changes can be reliably achieved remains a significant challenge. In very broad terms, when attempting to manipulate the microbiota, there are changes that are generally regarded as desirable; (i) maintenance or enhancement of overall microbiota complexity, (ii) increased abundance of genera such as the lactobacilli and genera that produce short-chain fatty acids, and (iii) reduced abundance of bacterial families, such as the

Enterobacteriaceae, that are known to include common pathogens. There are two main positive ways to manipulate microbiota composition; either by directly supplementing the microbiota with the addition of exogenous microbes, using probiotic products, or by changes and additions to the feed and water. There are other, potentially negative, influences on the gut microbiota, such as heat stress and breaks in biosecurity, which should be avoided to protect the microbiota. Probiotics are widely used in the poultry industry and many commercial products are available. There are a variety of potential mechanisms of action of probiotic strains – some of which may result in changes to the gut microbiota. All current products are recommended to be used regularly or continuously, indicating that their effects are short-lived. This indicates that most products probably don't have a lasting effect on the gut microbiota composition. Some probiotics have direct antimicrobial activity against pathogens, such as *Clostridium perfringens*, whereas others may produce beneficial outcomes by directly affecting host biology, for example by modulating the host immune system or by improving gut function in a variety of ways. Such direct effects on the host may result in secondary impacts on the gut microbiota. Various opportunities and limitations in probiotic identification, characterization, development, and use, will be discussed. Feed composition can have a profound effect on microbiota composition. However, the main ingredients of a diet are usually dictated by the price and availability of raw ingredients and so there is usually limited ability to change these. However, feed additives are widely used and many function via direct impacts on gut microbiota. Prebiotics (various forms of complex carbohydrates/fiber) can increase the abundance of beneficial bacteria. Other additives will be discussed that have been shown to either increase the abundance of beneficial bacteria or decrease the abundance of pathogens.

Key Words: microbiota, probiotic, prebiotic

463S Strategic modulation of intestinal microbiome: An immunologist perspective. Ryan Arsenault*; *Animal and Food Sciences, University of Delaware, Newark, Delaware, United States.*

Crosstalk between the immune system and the microbiota is an important, and understudied, consideration in designing microbiome modulation strategies. To understand how and why the immune system should be taking into account we must return to first principles regarding immunity and the microbiota. The role of the immune system is to prevent pathogens from entering the host; however, the evolution of the adaptive immune system has allowed the host exquisite control over our commensal microbiome. The host gains functional benefits from the microbiome and the myriad signals generated by these microbes allow for tissue development and full immunological potential. A microbiome intervention strategy must consider how this signal repertoire can be altered and what will be the most effective means of signaling for the change in host response

that is desired.

Key Words: immunity, gut, inflammation, signaling

464S Strategic modulation of intestinal microbiome: An allied industry research scientist perspective. Nadia Yacoubi*; *Nutrition and Care, Evonik Operations GmbH, Hanau-Wolfgang, Germany.*

Gut health plays a central role in modern poultry production as the gut is an essential organ for digestion of nutrients and defense against external invaders (pathogens, virus, parasites, toxins, etc.). The microbiome is a key component of the gut that has been investigated thoroughly in the last decade. Recent advances in molecular biology, new analytical methods, and big data analysis have made investigation of the microbiome easier and cheaper. This in turn has enabled microbiome research to become a strategic field for the poultry industry. Feed and feed additive companies are developing new nutritional solutions and concepts to maintain gut homeostasis in poultry by maintaining a well-balanced microbiome, a robust immune system and gut integrity. Recent research has shown that nutrition has a major impact on the gut microbiome composition and metabolism, as well as the interaction of the microbiome with the gut tissues and immune system. In the past, antibiotic growth promoters had the biggest impact on gut microbiome composition, but since the development of antibiotic free production, macro-nutrient composition of the feed (soluble and insoluble fibre, protein quantity and quality, etc.), micro-nutrients in the feed (minerals, vitamins) and feed additives (probiotics, prebiotics, organic acids, exogenous enzymes, etc.) are the most relevant microbiome modulators. In this talk the effect of feed composition, ingredient quality, nutritional value of the diet and the main feed additives on the gut microbiome will be discussed through examples from internal research and literature.

Key Words: Broiler, nutrition, Gut health, microbiome, Industry

465S Strategic modulation of intestinal microbiome: An extension specialist perspective. Audrey P. McElroy*; *Poultry Science Department, Texas A&M University, College Station, Texas, United States.*

Conducting pertinent and timely applied research, working in collaboration with stakeholders, and communicating the results to the poultry industry is an essential component of an Extension Specialist's role. The perspective of an Extension Specialist is more often on research that can create immediate impact through application in the industry. The intestinal environment including the mucosal structure and function is influenced by many factors including environmental management, embryonic incubation, dietary ingredients, protein level, composition, and quality among others. The health of the intestinal tract is extremely important as nutrient digestion and assimilation for growth occur here. Intestinal integrity is based on the system

functioning properly for digestion, absorption, secretion, and immunity. Subsequently, when disruption of this system occurs, major consequences in terms of bird health and growth follow. Maintaining intestinal integrity is a daily challenge in today's poultry industry, and it will likely become even more challenging with increasing environmental considerations, changing feed ingredients, and reduced medication usage. These increasing constraints demand Extension Specialists continually seek new opportunities or reconsider adopted strategies for maintaining a healthy and optimally functional intestinal mucosa that can be effectively applied in the industry.

Key Words: Intestinal health, extension, applied research

466S Strategic modulation of intestinal microbiome: A field nutritionist perspective. Alejandro Corzo*; *Aviagen, Huntsville, Alabama, United States.*

As a nutritionist of a primary breeder company, I spend considerable time visiting breeder flocks. As a result, my recent experiences are based mostly, but not exclusively, on the intestinal health that I observe in parent stock chicks, juveniles, pullets, hens and roosters. As a nutritionist, my observations rely on the collaboration with a field veterinarian during posting sessions, or pullet/breeder personnel with whom I visit flocks. Direct evaluation of gut health integrity is obtained from the posting sessions, whereas indirect gut health observations are made directly in the inside of the chicken house. For the latter, it becomes essential to focus on minor details and to employ my senses astutely. Valuable information can be gathered by listening to a flock, observing the characteristics of fecal and cecal droppings as well as the condition of the litter, and from those smells spotted around the house. In an age where antibiotics are not prophylactically used in internal feeds, my perspective of intestinal health focusses in evaluating those feed ingredients in a ration, and any potential they may have for disturbing gut harmony. In an integration, breeder feed costs represent less than 10% of the total feed cost, but in a primary breeder operation they are the overwhelming majority. Cost in pullet and breeder feed is not constantly scrutinized for "least cost", and rather centers on utilizing feed ingredients that are consistent and reasonably inexpensive. The concept of maximizing digestibility becomes secondary as most breeder feeds use diluents that result in nutrient and caloric density of lower values than that of broiler feeds. However, sometimes less nutrient dense feedstuffs can possess properties that can increase intestinal viscosity, motility, transit rate, malabsorption and create a scenario where dysbiosis could emerge. In summary, I would like to think that the perspective of a field nutritionist on the concept of gut health is that of an art based on collaborative efforts, rather than a science.

Key Words: Gut health, Dysbiosis

467S Strategic modulation of intestinal microbiome: A Field Veterinarian Perspective. Charles L. Hofacre*; *Poult. Sci. 100 (E-Suppl 1)*

Southern Poultry Research Group, Inc., Watkinsville, Georgia, United States.

Intestinal health is key to success in broiler production from many different perspectives and maintaining a healthy intestinal bacterial flora is key to maintaining a healthy intestine. Birds with an imbalance of the intestinal microflora may have a higher feed conversion, less uniform body weight and are more susceptible to secondary bacterial infections, such as *Clostridium perfringens* and *Salmonella* sp. In the past, it was much easier to maintain a healthy normal flora in broilers to prevent the growth of many pathogens by using low doses of antibiotics that are highly effective against gram positive bacteria like *Clostridium perfringens*. In addition to poultry disease prevention, Esco Numi, Geoff Mead and others have demonstrated that young chicks with healthy microbial flora will have enhanced resistance to colonization of *Salmonella*. In this paper, we will discuss the situation that can result in broilers having an intestinal microflora disruption and give examples of applied methods for enhancing or replacing the flora to a healthy balance.

Key Words: necrotic enteritis, intestinal flora, *Salmonella*, antibiotic use

468S Gut microbe interaction with pathogens: Opportunistic antagonism. Kristina M. Feye*; *Food Science, University of Arkansas, Fayetteville, Arkansas, United States.*

At the very least, we know that certain environmental conditions like inflammation can facilitate disease. Over the past few years, we have begun to recognize different avenues for impacting the environment of the microbiota and shifting the potential of the gut towards a less pathogen friendly space. In many ways, we as a field believe that is by producing favorable, "probiotic" like shifts in the microbiota. However, there may be other ecologically driven factors that enable improved survivability over an entire population of organisms. This talk will concatenate some of the microbiota changes associated with pathogen exposure in both native and experimental infections as measured by 16S Illumina MiSeq sequencing. Multiple trials will be compared and trends will be discussed. Ultimately, evidence suggests that the dispersion of the microbial communities across a population of birds and the ability to resist that change may serve to be more beneficial as a whole to a flock as compared to individual changes within the microbiota.

Key Words: Microbiota, gut health, pathogens

469S Gut microbial interaction with the host: Education and adaptation. Vanessa Leone*; *Department of Animal & Dairy Sciences, University of Wisconsin, Madison, Wisconsin, United States.*

Host-microbe interactions are key elements in poultry production that influence flock health, welfare, and production outcomes, including FCR. Acquisition of a

microbiome begins at the time of hatch. Initial microbial colonizers are heavily influenced by both bird intrinsic, i.e., genetics, breed, and sex as well as bird extrinsic factors including farm management strategies, i.e., feed, stress, and health interventions. Both the type and abundances of specific microbes acquired post-hatch can serve as a barrier to competitively exclude enteric pathogens as well as dramatically shape the host immune system, which persists throughout the life cycle of the bird. Microbial colonization occurs in a non-stochastic, successive fashion with corresponding changes in host immune cytokines, where stability, or maturation of the microbiota is achieved by ~3 weeks post-hatch. While inflammatory events induced by the acquisition of microorganisms in early life are critical for education of innate and adaptive immune responses, dysregulated inflammation can have devastating consequences for bird health, welfare, reproduction, and growth. The acquisition of gut microbes is impacted by many factors in the production setting, however, little is known about what results in a “healthy” microbiome, in part due to the heterogeneity across production systems. Patterns of adaptation, competitive interactions, cooperation, and synergy between initial microorganisms in the context of mucosal immune development is paramount to understanding poultry gut ecosystems. It is unlikely that a “one-size-fits-all” approach will be identified to prevent breakdown of the delicate balance that drives host-microbe synergism vs. antagonism. More than likely multi-prong approaches are needed to maintain these critical interactions over the lifespan of the bird. Gaining more mechanistic insight into complex host-microbe crosstalk during early life events will allow for the emergence of novel strategies that maintain appropriate host immune function in the face of a multitude of challenges faced in the modern poultry production setting.

Key Words: Microbiome, Immune function, gut health, performance, immune adaptation

470S Feed additives: Manipulating gut microbial interactions. Dana K. Dittoe*; *Animal and Dairy Sciences, University of Wisconsin, Madison, Wisconsin, United States.*

With the use of antibiotic free and no antibiotic ever (NAE) poultry production systems, there has been an increase incidence and prevalence of dysbacteriosis and disease within poultry. As mortalities occurring from disease can contribute between 10 to 20% of production cost in developed countries, there is need to develop effective alternative strategies to the prophylactic use of antibiotics. Currently, numerous feed supplements are being proposed as effective antibiotic alternatives in poultry diets, such as prebiotics, probiotics, acidic compounds, competitive exclusion products, herbs, essential oils, and bacteriophages. These feed additives primarily work by altering the physiological and morphological structure, inflammatory response, and the microbiota composition of the gastrointestinal tract (GIT) both directly and indirectly.

This in turn has the potential to reduce pathogenic bacteria and parasites living within the GIT and improving bird performance. However, each antibiotic alternative may function differently and therefore generate a different GIT microbiota composition. Thus, there may be optimal strategies for utilizing specific alternatives during different rearing phase and in different poultry sectors (meat versus egg production). Therefore, this symposium aims to provide an understanding of the effects these alternative feed supplements have on the microbiota of poultry and the effect those changes have on the prevalence of pathogens and diseases in poultry.

Key Words: feed additives, poultry, gastrointestinal tract, microbiota

471S Gut metabolic crosstalk: Microbes feeding microbes. Steven C. Ricke*; *Department of Animal and Dairy Sciences, University of Wisconsin, Madison, Wisconsin, United States.*

Much of the focus in understanding the poultry gastrointestinal tract (GIT) has emphasized compositional analyses of the microbiota. Using microbiome 16S rDNA sequencing taxonomy identification and assessment of microbial GIT population diversity have been the bioinformatic metrics commonly employed to characterize the GIT microbial ecology. These bioinformatic metrics serve to identify members of the GIT microbial population as well as compare different GIT populations for detectable differences. This information has considerable utility for assessing the impact of a number of factors such as bird age, feed additives, and the presence or absence of pathogens on the GIT microbial consortia. However, it offers only limited information on GIT microbial functionality. Other analyses such as metabolomics are needed to delineate utilization of substrates present in the GIT for microbial growth and fermentation into various end products such as volatile fatty acids. Not surprisingly even when minimal GIT microbial compositional differences occur in response to a particular dietary treatment or environmental condition, considerable differences in fermentation activity can still be detected. This can be further complicated by the fact that end products produced by some GIT microorganisms can serve as substrates for other members of the GIT microbial community. This is referred to as metabolic cross feeding or cross talk and can involve numerous microorganisms within the GIT microbial community including foodborne pathogens. This presentation will discuss some of the types of cross feeding that may occur and the implications for foodborne pathogen interaction with the GIT microbial community.

Key Words: Gastrointestinal tract, fermentation, metabolic cross talk, foodborne pathogens

472S Managing amino acid nutrition in reduced crude protein diets: A swine nutritionist’s perspective. Jason Woodworth*, Mike Tokach, Joel DeRouchey, Jordan Gebhardt, Robert Goodband; *Kansas State University,*

Manhattan, Kansas, United States.

Increased availability and cost competitiveness of synthetic amino acids (AA) has allowed swine nutritionists to formulate diets with reduced crude protein (CP) compared to more typical formulations of the past. Advantages of lower CP diets include less nitrogen excretion, lower diet costs, and less nitrogen substrate available for proliferation of pathogenic bacteria leading to lower post-weaning diarrhea (PWD). Swine diets often contain feed grade lysine, threonine, methionine, tryptophan, valine, and isoleucine. While the histidine requirement for growing pigs has been established, feed grade histidine is not readily available at this time. Maintaining the SID Lys:CP ratio to be at or below 6.35 helps nutritionists ensure there is enough nitrogen for endogenous non-essential AA production. In post-weaning diets, reducing CP to approximately 18% (from a typical 21-22%) by using feed grade AA-supplemented diets allows for reduced PWD without negatively impacting growth performance. Opportunities to reduce SID Lys in early post-wean diets and taking advantage of compensatory gain in later phases is a routine strategy to control PWD while maintaining nursery ending weights. In finishing pigs, high levels of feed grade AA are utilized to lower CP with data suggesting at least 12% CP needed to maintain non-essential AA production (Soto et al., 2019). Recent AA modeling to predict growth performance (Cemin et al., 2019) has defined important relationships between branch-chain AA as well as large-neutral AA (Trp) such that when high levels of Leu are present in diets, increased Val, Ile, and Trp are needed to maintain growth performance. Subsequent research (Kerkaert et al., 2021) has confirmed the accuracy of the models and illustrated that increased Val, Ile, and/or Trp added at levels above normally accepted levels to support growth can ameliorate some of the poorer performance observed when diets contain high levels of Leu. Similar models have been developed for lactating sows to predict nursing litter weight gain based on branch-chain AA and large-neutral AA relationships. Swine nutritionists are expected to continue to focus on utilizing increased levels of feed grade AA and reducing CP as a means to control diet cost and optimize growth performance and health status of pigs.

Key Words: Swine, Crude Protein, Amino Acids, Branch-Chain Amino Acids

473S Understanding the branched-chain amino acid conundrum and how to use it to maximize performance.

Michael T. Kidd*¹, Ed Gbur, Jr.², Craig W. Maynard¹, Justina V. Caldas³, Jason T. Lee⁴; ¹*Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States*, ²*Agricultural Statistics Laboratory, University of Arkansas, Fayetteville, Arkansas, United States*, ³*Aviagen, Inc., Huntsville, Alabama, United States*, ⁴*CJ America – Bio Business, Downers Grove, Illinois, United States*.

Feed grade acceptability of L-Val in poultry feed formulation is increasing, and as a result, meeting least cost Poult. Sci. 100 (E-Suppl 1)

precision nutrition is allowing for reduced amino acid and nitrogen excesses. Each formulation acceptance of the next limiting amino acid in fed grade form entering the ingredient list results in diets with lower oilseeds and increased cereals, of which is of particular interest in corn-based diets that are high in Leu. Further, diets containing L-Val result in increased pressure on the nutrient minimums for Ile and Arg. Published broiler research evaluating singular responses of Val, Ile, or Leu and combined responses of the branched-chain amino acids were assessed and compared. Test diets from manuscripts selected were recreated. Subsequent levels and ratios of Val, Ile, and Leu were assessed for significance on live performance and breast meat yield in a meta-analysis. Ideal protein ratios (i.e., Val/Lys and Leu/Lys) were good predictors of live performance and breast yield responses, as well a combined ratio (i.e., (Val+Ile)/Leu) that resulted in good predictability. The uniqueness of the branched-chain amino acids in poultry nutrition may necessitate their individual nutrient minimums conditioned by dietary levels of one another.

Key Words: Branched-chain amino acids, Broiler, Isoleucine, Leucine, Valine

474S Dietary protein level and source and gut health in broilers. Alfons Jansman*; *Animal Nutrition, Wageningen Livestock Research, Wageningen, Netherlands.*

The traditional focus on the use of protein sources as ingredient for broilers diets has been on the provision of essential and non-essential amino acids to birds as building stone for body protein synthesis and related growth performance. Over the past decade, for sustainability reasons, increasing emphasis has been put on lowering dietary protein level while maintaining growth performance by supplementing essential amino acids in free form to the diets. Together with the genetic improvement over the years, this approach allowed to increase N-efficiency in broilers and reduce the environmental excretion of N into the litter and the environment. Over the last years, however, traditional and novel protein sources have also been judged from other perspectives apart from their nutritional value e.g. in relation to specific effects on intestinal development and gut functionality. Ingredients having specific prebiotic or antimicrobial properties are examples of such protein sources. The latter relates to the presence and activity of a complex microbiome in the gut of broilers which contributes to the barrier function of the gut and also supports health and functionality of the gut. Enhanced fermentation of protein in the gut increases the concentrations of e.g. ammonia and biogenic amines in the gut which are a threat for proper functioning of the gut. This indicates that not only the content of enzymatic digestible protein in dietary protein sources is of importance but presence of potentially fermentable protein in the gut as well. The ratio between the both protein fractions in protein sources is also dependent on the kinetics of protein digestion in the digestive tract, which can differ widely between

sources. Protein fermentation also contributes to wet litter problems and release of protein fermentation products. High protein diets and diets containing a high proportion of fermentable protein can stimulate the growth of potential pathogenic species such *Clostridium perfringens*, contributing to the occurrence of necrotic enteritis, still an important gut disease in broilers. In conclusion, protein nutrition of broilers requires an integrative approach including provision of essential and non-essential amino acids and consideration of effects on gut health and functionality by the presence of specific functional or antinutritional constituents in protein sources.

Key Words: protein source, protein level, gut health, broilers

475S Industry Perspective: Using branched-chain amino acids to improve performance. Chance Williams*; *Wayne Farms LLC, Hoschton, Georgia, United States.*

In the past, the use of synthetic L-valine or L-isoleucine in commercial broiler formulas has primarily been lacking due to the absence of a feed cost benefit. Therefore, altering digestible ratios of valine or isoleucine to lysine was done so through protein-contributing ingredients (i.e., soybean meal or animal protein meal), albeit at the potential detriment of various factors besides increased feed cost. In recent years, however, greater volumes of L- valine and L-isoleucine have entered commercial formulas primarily from improved market pricing. The use of these synthetic amino acids has given nutritionists the ability to further balance the amino acid profile, as well as approach the interrelationships of branched-chain amino acid (valine, isoleucine and leucine) ratios and reduced protein diets.

Key Words: Broiler, Valine, Isoleucine, Branched-chain

476S Improving economic and environmental sustainability of poultry production using an innovative precision feeding system. Bertrand Meda*¹, Eva Pampouille², Yann Guyot²; ¹*INRAE UMR BOA, Nouzilly, France*, ²*ITAVI, Nouzilly, France.*

Precision feeding in broiler production could help reducing feeding cost and nutrient excretion, thus improving the sustainability of the poultry sector. However, in commercial farms, individual feeding is not a realistic option due to the large number of animals. Therefore, precision feeding could be defined as “delivering the proper diet at the right moment for the flock”, or in other words, dynamically adjusting feed composition to requirements at the flock level. This can be achieved by blending two different diets to compose an “optimal diet” for a given day. The first step to this approach is to assess the “most probable” body weight (BW) gain for the day to come. To do so, BW data measured by automatic scales can be used, such devices being nowadays widely used in commercial farms. On a given day (D-day), BW data from previous days can thus be used to fit a $BW=f(\text{age})$ model, from which BW gain for D- day can be estimated. The second step is to provide an estimate of the average

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requirements of the flock. Protein and fat deposition associated to BW and BW gain (model inputs) can be firstly predicted with allometric equations, from which, growth and maintenance requirements for metabolizable energy, digestible lysine, and available phosphorus can be derived. The last step is to estimate the composition of the “optimal diet” using daily requirements and the nutritional characteristics of two “pre-diets” A and B to be blended in different proportions each day. From a practical point of view, the weighing and blending of the pre-diets as well as the distribution of the blend can be performed using a weighing-mixing hopper placed between the feed silos and the feeding line. In order to improve the economic robustness of the approach, the composition of the two pre-diets can be simultaneously optimized using a mathematical method called “bilinear formulation”. A first proof of concept for this approach has been carried out in experimental conditions using fast-growing broilers fed between 10 and 31 days of age. At slaughter, no statistical difference in BW was observed between control (C) and precision-fed (PF) birds, even though a significantly higher feed conversion ratio was found for PF birds compared to C ones (+0.029 point). However, overall feeding cost and phosphorus excretion were found to be significantly lower by about 8% and 12% in the PF strategy. These promising results demonstrate the potential of precision feeding for broilers, but many technical and practical issues still need to be considered for this strategy to be deployed in commercial farms (return on investment, pellets sorting by birds, implementation of models and decision rules in farm equipment...).

Key Words: precision feeding, modeling, requirements, excretion, feeding cost

477S Using in-house robots to monitor, detect and respond to real time issues. Colin Usher*; *Aerospace Transportation Advanced Systems Lab Food Processing Technology Division, Georgia Tech Research Institute, Atlanta, Georgia, United States.*

Researchers at the Georgia Tech Research Institute have developed and tested a ground-based robotic system capable of navigating and manipulating commercial poultry house environments. This robot is an intelligent system equipped with a suite of sensors including environmental sensors, 2D and 3D cameras, and a small robotic arm. The robot has been programmed to interact with chickens allowing it to safely navigate the environments without harming the chickens. This unique capability also gives the robot the ability to actively sense and characterize bird status as it moves through the environment. This talk will discuss potential ways that such systems can monitor, detect, and respond to several different types of issues in real-time and what perceived benefits can be achieved.

Key Words: Robotics, flock management, Automation, assistant

478S How advances in technology are making the

application of artificial intelligence and machine learning to poultry affordable, accessible and valuable.

Claire Lewis*; *Agri-Food, Harper Adams University, Royston, United Kingdom.*

This presentation will provide an overview on process technologies that involve cloud computing, IoT devices, and advanced data analytics that enables artificial intelligence and machine learning to become affordable and accessible in poultry production. There is a need to discuss and highlight the offering of such technologies against the current state of the art in poultry production. Whilst 'cool', are such technologies really necessary? Examples show, with promising results, how new technologies are being applied to commercial poultry production to monitor growth rate, uniformity, and bird behaviour. The benefits of these technologies are continually evolving, but fundamentally there is real potential to deliver 24/7 monitoring, information, and alerts that reduce labour requirements and ultimately enable producers to better focus their available resources on maximizing flock performance. Additional benefits include better insight on culling targets, reduced biosecurity risks, and less bird and worker stress.

Key Words: Artificial Intelligence, machine learning, technologies

479S Big data: What is it, what's meaningful, and how do we use it to design and implement new strategies?

Tammy A. Baltzley*; *Kreher's Farm Fresh Eggs, Clarence, New York, United States.*

Most producers collect production data on a routine basis. They are of course analyzing this data for indications of problems, trends, assessment of husbandry procedures, and financial benchmarks. Within the layer industry and most poultry segments there are key parameters that are routinely measured. These are critically important, but what if we combine those more routine benchmarks with some less often analyzed parameters. Big data or extremely large data sets can be analyzed using up to the minute data analytic tools and innovative flock management software to allow you to make production management decisions with the most complete picture possible.

Key Words: Data, Layer, Production, Management

480S Developing and maintaining student interest in a global pandemic. Elizabeth Karcher*; *Animal Sciences, Purdue University, West Lafayette, Indiana, United States.*

In 2020, classrooms across the country abruptly transitioned to emergency remote learning in response to COVID-19. Instructors quickly searched for guidance on ways to present course material in an online format that would still allow for course learning outcomes to be met. Perhaps the greatest challenge cited by instructors was engaging students when face-to-face meetings were not an option. This becomes an even greater challenge in poultry and animal science curricula that encourage hands-on learning. Most first year students are unaware of the opportunities in

the animal agricultural industries and engagement in the classroom is one way to spark curiosity and interest in the subject matter. But how can we accomplish this in remote learning? This presentation will focus on evidence-based remote learning strategies to develop student interest in and awareness of the animal agricultural industries.

Key Words: Curiosity, Engagement, Interest, Remote learning, Undergraduate

481S Creating online engagement in a world of black boxes. Dawn Koltes*, Anna K. Johnson, Peggy Auwerda, Laura Greiner, Sherrilyn Olsen, Christen Burgett; *Animal Science, Iowa State University, Ames, Iowa, United States.*

A global pandemic was declared on March 11, 2020 by the World Health Organization in response to a novel coronavirus (COVID-19). Iowa State University (ISU), like other academic institutions, transitioned suddenly to online instruction leading to a situation best characterized by a paraphrased quote from Neil deGrasse Tyson, "Learning is like...you know, you have to take your medicine. And that is what it has become. And that is unfortunate". Many students and instructors due to COVID were unable to connect live and this altered our engagement options and course modality. Compounding this were financial constraints, internet connectivity issues, and family care challenges. Therefore, this abstract explores the topic of engagement that will be discussed as three themes (a) engagement strategies, (b) engagement implementation considerations, and (c) academic community needs. Engagement strategies: Online learning altered the ways students engaged with class materials and relationships between peers, instructor(s) and teaching assistants. Successful engagement strategies have included shorter videos, less voice over powerpoint lectures, and diversifying assignments such as worksheets, quizzes, or projects to increase and support student learning. We, also, learned that peer-to-peer engagement was an important aspect in a live classroom, which was missing online. To increase these interactions, written or video discussions in the form of small groups or discussion boards were used to guide, educate, and facilitate conversation. Engagement implementation considerations: While strategies for effective, impactful, and meaningful engagement depends upon the instructor's definition of successful student engagement, several aspects should be considered. When adding a new strategy to a course, one should reflect on how the strategy fits within the course objectives, delivery mode, grading, how to deal with technical issues, and lastly, time needed to design/develop and/or refine the activity for current- and future use. Academic community needs: Within the Department of Animal Science at ISU, we recognized that educational videos, and fee-based programs were not publicly available and became difficult or impossible to generate during the pandemic. Database development with created resources and tools would be a great asset for academic use and provide collaboration among institutions of higher education across the country.

In conclusion, while the global pandemic altered the way we taught animal science, instructor innovation in our department led to unique and successful strategies to engage students.

Key Words: student engagement, virtual learning, Curriculum

482S Hands-on learning from a distance: Hybrid and virtual lab experiences. Benjamin Wenner*; *Animal Sciences, The Ohio State University, Columbus, Ohio, United States.*

An essential aspect to education in animal sciences is the engagement of students through hands-on activities to reinforce course content. However, the post-pandemic world limits in-person interaction and threatens to undermine the essential laboratory instruction. As students return to in-person activities, instructors are faced with the challenge to engage each student at their own comfort level with face-to-face interaction. This presentation will integrate the wins and losses from the past year's lab courses to creatively approach the next challenge: hybrid and virtual labs.

Key Words: laboratory, student engagement, hybrid learning

483S Modeling necrotic enteritis: Applying lessons learned. Lisa R. Bielke*, Audrey F. Duff, Kaylin Chasser, Whitney Briggs, Kim Wilson; *Animal Sciences, Ohio State University, Wooster, Ohio, United States.*

Necrotic enteritis (NE) is a disease that has gained increasing relevance in the poultry industry with effects on body weight, uniformity, and livability. As scientists study prophylactic and therapeutic strategies to control NE, modeling predisposing factors and disease induction within research settings is a critical component to consider. As a multifactorial disease, research related to NE is filled with a variety of methods, which often leads to contradictory results between laboratories. As a disease of the gastrointestinal tract, research models generally disrupt gut homeostasis to create conditions favorable for overgrowth of *Clostridium perfringens*. Commonly, this is achieved by varied combinations of altering diet composition and introduction of infectious agents. While some methods not consistent with commercial production practices can lead to important information regarding pathogenesis, development of intervention strategies should include commercial conventions while avoiding outdated or inapplicable methods. Numerous factors need to be considered when designing experiments to test disease control strategies. Bacterial strain selection can critically alter disease, especially with varying toxin production from isolate to isolate. Alpha-toxin and NetB are major enzymes associated with NE and their production within the gut can result in varying clinical signs, lesions, and changes to growth performance. Severity of disease can be adjusted in most research models by altering the degree to which

predisposing factors are applied, which is an important consideration for experimental design. While sub-clinical NE is perhaps a larger concern to integrators than clinical disease, detecting significant treatment effects within a sub-clinical research model can be difficult. However, in experiments that include heavy clinical disease such as high mortality, the severity of disease may be too difficult to overcome. Finally, when testing new tools against NE, mechanism of action should be considered as part of the experimental design, especially in relation to prophylactic, therapeutic, and recovery stages of the disease. Each of these components can have effects on the outcome of experiments, and scientists need to consider long term goals and industry applicability as they study NE mitigation strategies.

Key Words: necrotic enteritis, alpha-toxin, *Clostridium perfringens*, NetB, models

484S Effects of the housing environment on necrotic enteritis. Jeremiah D. Davis*; *National Poultry Technology Center, Auburn University, Auburn, Alabama, United States.*

Managing necrotic enteritis has been challenging on commercial farms as the industry has moved to antibiotic-free (ABF) production. This presentation will discuss challenges and successes of managing necrotic enteritis on farms.

Key Words: necrotic enteritis, housing environment, litter moisture

485S The cocci conundrum. Charles L. Hofacre*¹, Greg F. Mathis²; ¹*Southern Poultry Research Group, Inc., Watkinsville, Georgia, United States,* ²*Southern Poultry Research, Athens, Georgia, United States.*

The poultry industry has been facing multiple new challenges in bird health, animal welfare and food safety. Many of these challenges are inter-related, for example, poor bird intestinal health can result in wet litter which can affect foot pad quality and give *Salmonella* an opportunity to proliferate in wetter litter. One of the major bird health issues that can instigate this is the protozoa *Eimeria*. *Coccidia* in broiler chickens are ubiquitous in broiler houses around the world; so their control becomes imperative for successful broiler production. Of the *Eimeria* species that infect broilers, *E. maxima* is the primary insult that triggers the normal flora bacteria *Clostridium perfringens* to grow and produce toxins that results in necrotic enteritis. As more broiler companies market antibiotic free chicken, there are less choices for control of *coccidia*. Also, the chemical anticoccidial drugs have been used for more than 40 years, so *coccidia* resistance can also be an issue. This leaves us with the option of *coccidia* vaccination (controlled exposure), phytogenic products with anticoccidial properties, chemical anticoccidial drugs alone or combinations of vaccines with the chemical or phytogenic products. (bioshuttles) This paper will discuss how *Eimeria*

maxima and *Clostridium perfringens* result in necrotic enteritis and how we can successfully manage these two pathogens so we maintain bird health, welfare and provide a safe food.

Key Words: *Eimeria maxima*, *Clostridium perfringens*, necrotic enteritis

486S Managing necrotic enteritis in commercial broiler production: What can nutrition do? Regis F. Pastorelo Meurer*; *Pilgrims, Longview, Texas, United States.*

The success of high productivity in the poultry business depends, among other factors, on the adequate absorption of nutrients present in the diet. For this, the intestinal epithelium of birds must present integral morphophysiological characteristics, since it is the main site of digestion and absorption of nutrients. One of the diseases that directly affect intestinal integrity and that causes one of the biggest economic losses for the poultry industry is Necrotic Enteritis. It is estimated that about 40% of commercial broiler flocks have some degree of this disease. Necrotic Enteritis is a subclinical or clinical form of infection in poultry caused by toxins released by *Clostridium perfringens*, a sporulated gram-positive bacteria considered a member of the normal intestinal microbiota. Most of the time, *Clostridium perfringens* is present in the cecae of birds without causing disease. However, some factors can trigger an increase in the number of this bacteria, promoting a greater production of toxins. Among the most common predisposing factors for the release of toxins are environmental factors, coccidiosis, mycotoxins, diets with high levels of non-starch polysaccharides or with high protein levels, and contamination of ingredients used in the feed. In subclinical cases, these toxins cause intestinal necrosis with hemorrhage and extensive damage to the intestinal epithelium, leading to decreased digestion and absorption of nutrients, with reduced weight gain and increased rate of feed conversion. In acute clinical cases, there is a significant increase of mortality, causing an even more severe economic loss. Although in-feed antibiotics may effectively control most cases of Necrotic Enteritis, restrictions on the use of antibiotics have changed the approach to controlling this disease. Current strategies focus on managing predisposing factors. In this context, the role of the nutritionist should be to avoid or reduce the damage caused by *Clostridium perfringens*, acting in advance on the quality of the ingredients or with different strategies in the formulation of the diets, or even using additives available to minimize the impact caused by Necrotic Enteritis.

Key Words: Necrotic Enteritis, *Clostridium perfringens*, Broilers, Nutrition

487S If you have people skills AND can handle data...the poultry industry needs you. Bruce Stewart-Brown*; *Perdue Farms Inc., Salisbury, Maryland, United States.*

The data generated in the Poultry Industry is impressive. Numbers are everywhere. In the next 10 years there will be more. It all means something and much of it is related. However, much of the opportunity can go wasted if we don't find the people to study and analyze it. At the same time, don't miss the opportunity to develop your personality and your people skills. Working with others is a skill that requires practice. Jobs that need these skills include Marketing, Food Safety and Quality, Live Operations, Plant Operations, Supply Chain Management, and Research and Development.

Key Words: Marketing, Food Safety and Quality, Operations, Supply Chain Management, Research and Development

488S Opportunities in the field of poultry nutrition and allied industry. April Waguespack Levy*; *Animal, Nutrition and Health, DSM Nutritional Products, Ankeny, Iowa, United States.*

The field of poultry nutrition (from live production, allied industry, academia or government) offers a wide range of experiences and opportunities. Allied in particular interacts with every corner of the industry with work in diet formulation, research trials, new product development and regulatory as well as emerging areas of opportunity in sustainability through nutritional solutions. Prepare yourself through internships for exposure, industry conferences for networking, and strong class selection tailored to the roles and experiences that most interest you.

Key Words: nutrition, allied industry, research, formulation

489S Career opportunities in academia. David J. Caldwell*; *Department of Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States.*

Six independent poultry science departments exist in the United States currently. Throughout the U.S. and Canada, more than 20 additional academic departments are home to faculty members who conduct research, offer courses, or deliver extension/outreach programs specific to the field of poultry science. Collectively, these academic programs make significant contributions to the employment and staffing needs of the commercial poultry industry and the allied industry. The success and viability of these programs is essential to our discipline. Abundant opportunities exist in academia for individuals interested in pursuing careers in research, teaching, and/or extension and outreach in poultry science.

Key Words: academia, research, teaching, extension, outreach

490S Balancing feed quality, nutrition, and hygienics. Joseph S. Moritz*; *West Virginia University, Morgantown, West Virginia, United States.*

The value of feeding high quality pellets to meat birds has been debated for decades. There are integrators, managers,

and nutritionists that support investment in feed manufacture equipment and time to create pellets of high quality to optimize bird performance and others that believe that simple conveyance of feed through a conditioner and pellet mill at a high rate is adequate to improve nutrition and bird performance. The reality may be that due to the multitude of variables associated with feed manufacture such as conditioning temperature, conditioning time, production rate, steam quality, etc. and the unique conditions experienced by each mill such as ingredient quality, equipment, and ambient conditions both theories are correct. The pelleting process utilizes saturated steam to convey heat and moisture to ground ingredients for a determined temperature and time during conditioning with subsequent frictional heat and pressure during pellet die extrusion to ultimately form a pellet. These thermal processes enable pellet binding reactions such as starch gelatinization and protein gelation, but can also effect heat labile feed components such as trypsin inhibitor complexes, amino acids, vitamins, and exogenous enzymes that may increase or decrease nutrient availability. In addition, temperature and time combinations associated with pelleting can kill feed pathogens. A few, recent publications that highlight pelleted feed quality, nutrient availability, and hygienics will be reviewed. These data provide insight to why a comprehensive approach should be considered when determining feed manufacture technique to optimize bird performance.

Key Words: Pellet, amino acid, digestibility, hygienics

491S Maintaining a balance between feed quality, nutrient digestibility, and production goals. Wilmer J. Pacheco*; *Poultry Science, Auburn University, Auburn, Alabama, United States.*

Improvements in genetic selection and nutrition have allowed broilers to reach market weight in shorter time with less feed needed per gram of weight gain. Since feed is typically intended to be fed as a sole source of nutrients, it is critical to ensure birds receive all nutrients in each bite. The feed industry has evolved over the years from water powered mills to fully automatized feed mills. Although the principles of feed manufacturing have remained rather constant, new technologies have become widely available. The use of variable frequency drives has become an option to modify corn particle size by adjusting the tip speed of the hammers in hammermills. The presence of coarse particles in the microstructure of the pellets increases reverse peristalsis, nutrient digestibility, and feed efficiency of birds. Among feed milling processes, conditioning has been designed to efficiently increase heat and moisture migration into the core of feed particles to improve pellet quality. However, a balance between temperature and retention time must be attained to protect the stability of heat labile nutrients and exogenous enzymes. After conditioning, the conditioned mash is pressed through a pellet die consisting of multiple openings, which aids in the formation of wet bonds between particles and starch gelatinization in the

surface of pellet, contributing to pellet durability. During cooling, solid bonds are formed between feed particles and soluble components are recrystallized. Additionally, it is important to understand the relationship between air flow and retention time to control moisture and temperature in the finished feed. In general, it is important to maintain a balance between production and feed quality goals, which can be accomplished via employee training and equipment maintenance. Management strategies must be adjusted depending on each particular situation. For instance, if pellet quality is greater than 90%, but employees have to work longer hours leaving no time for maintenance, increasing the amount of fat addition in the mixer by 0.25% or reducing die thickness by ~10%, can increase production rate. Contrary, if pellet quality is marginal, but the feed mill is running shorter hours and continually changing feed types due to low finished feed capacity, reducing mixer fat can improve pellet quality. Overall, changes in production rate merit modifications in cooling strategies as well as in the evaluation of pellet quality. If production rate increases, pellet depth in the cooler could be increased to maintain a comparable retention time for proper cooling. In summary, it is of great importance to maintain a balance between production goals, feed quality, and poultry performance.

Key Words: conditioning, pelleting, cooling, pellet quality, production goals

492S The quest for optimal pellets, with an emphasis on nutritional value. M. Reza Abdollahi*; *School of Agriculture and Environment, Massey University, Palmerston North, New Zealand.*

To achieve the genetic potential of modern meat-type birds, feed intake must be tightly monitored and maximized. Currently, majority of the feed used in the production of broilers is fed in pelleted form. There are several mechanisms that underpin the advantages of pellet feeding over mash diets, but the foremost factor is simply the increased feed intake. Though the nature of digestibility response to pelleting is dependent on the ingredient and the specific nutrient, recent evidence suggests that pelleting has no positive impact on the digestibility of major nutrients in cereal-based broiler diets. Moreover, the efficiency of feeding pelleted feed to broilers in determining the actual performance responses depends on the dietary nutrient density, and nutrient availability which, in turn, is influenced by grain type, and processing variables such as particle size reduction and conditioning temperature. To maximize pelleting benefits, it is critical to decide which level of nutrient density should be used and to identify manufacturing techniques to create high quality pellets that are highly digestible. Based on available data, it is also reasonable to assume that nutrient requirements of modern broilers and in vivo feed ingredient evaluation may depend on feed form and that these data should be generated using pelleted diets to resemble the feeding practice in industry.

Key Words: Broilers, Pelleting, Nutrient digestibility, Particle size, Conditioning temperature

493S Impact of physical feed quality on meat bird performance. Kelley G. S. Wamsley*; *Mississippi State University, Mississippi State, Mississippi, United States.*

Utilizing pelleted diets is a standard practice in the commercial meat bird industry due to associated improvements in performance. In general, improved physical feed quality translates into improved performance. However, it is important to note that the magnitude of this improvement may be influenced by a variety of factors such as genetic strain, length of the desired grow out, and dietary phase. Additionally, a multitude of factors can influence feed form, nutrient availability, as well as bird performance (e.g., production rate, ingredient particle size, conditioning temperature, diet formulation, cooling, and pellet die specifications); thereby confounding data, making it difficult for meat bird producers to fully realize the implications of investing in feed quality. However, this also reveals the critical/complex role of feed form/physical feed quality in improving overall meat bird performance and processing characteristics. Ultimately, data demonstrates that feeding improved physical feed quality (even modest improvements) has economic importance as long as nutrition is not compromised.

Key Words: meat bird performance, physical feed quality, pellet quality, feed form

494S Xiant Technologies PAWS™ illuminating the rhythm of life. Daren Suntych*; *Xiant Technologies, Greeley, Colorado, United States.*

Xiant Technologies (XTI) has created a highly differentiated approach to agricultural lighting for both Plants and Animals. XTI uses light as a primary controller of biological function by targeting specific photoreceptors. Through dosing of pulses of alternating wavelengths of light in specific patterns to target specific biphasic photoreceptors Xiant's PAWS™ can become the fundamental controller of chromophore activity. By stimulating these latching and resetting chromophores, Xiant Technologies has been able to open a new paradigm in research and production. This not only increases organism livability and welfare it also maximizes an organism's growth and production capacity by providing light only when and how the organism is capable of utilizing it.

Key Words: photo receptors, chromophores, wavelengths

495S Photoreception in the duck: Even the brain has eyes! Gregory S. Fraley*; *Animal Sciences, Purdue University, West Lafayette, Indiana, United States.*

Lighting has profound effects on the physiology and welfare of all poultry species, and the duck is no exception. Ducks are seasonal breeders and as such require supplemental

lighting to maintain fertility. We know from past research that the eyes or pineal gland do not participate in light responsiveness for gonadal development or recrudescence. Another set of photoreceptors exist within the diencephalon referred to as deep brain photoreceptors (DBP). Past research has demonstrated relationships among lighting, deep brain photoreceptors, and reproductive fitness in ducks. The purpose of this symposium talk is to first provide an overview of the DBP system in general, and what is known for ducks specifically. Second, recent data will be discussed on the embryonic ontogeny of two of the DBPs in relation to retinal photoreceptors, and continue with the posthatch ontogeny of these two DBPs in relation to the onset of puberty. We will show data that suggests that one of the DBPs, melanopsin, may have a physiological role beyond reproduction. Finally, we will present data on a novel retinal photoreceptor, cryptochrome 2, that may have a unique role in visual perception. Although the duck will be highlighted observations can be translated to other poultry species.

Key Words: opsin 4, opsin 5, cryptochrome 2, development

496S Wait! Light can change the eye? Karen Schwean-Lardner*¹, Bruna Maria Remonato Franco²; ¹*Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada,* ²*Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.*

Alterations to lighting programs (duration and pattern of light and dark, intensity and wavelength) can have significant impacts on many productivity, health and welfare parameters, including growth, feed efficiency, mortality, mobility, lesions, and physiological and behavioural rhythms. Less attention has been placed on how light affects the eye and vision. The eye grows in a circadian pattern. Therefore, lack of diurnal patterns in photoperiod/scotoperiod, or lack of differentiation of day/night with respect to intensity, can disrupt the normal growth of the eye. This can result in abnormal shapes and, in some cases, the development of glaucoma. Although wavelength does not appear to cause these changes, myopia or hyperopia and alterations on spatial vision may result when various wavelengths are used in production systems. This presentation will focus on the recent literature regarding ocular health and light.

Key Words: Light, Vision, Ocular

497S How do birds see their world? Implications for poultry species. Esteban Fernandez-Juricic*; *Biological Sciences, Purdue University, West Lafayette, Indiana, United States.*

Birds are visually-oriented organisms, like humans are. However, the visual systems of birds and humans have some fundamental differences. In this presentation, I will review the main differences between bird and human vision from a physiological point of view and how these visual

perception differences may affect bird behavior, particularly that of poultry species. Relative to humans, birds can see more colors, discriminate between colors better, process visual information faster, and integrate visual inputs from different parts of their lateralized visual fields. The implication is that what is visually conspicuous for a human might not be for a bird, and vice versa. Additionally, the spectral properties of ambient light can modify the visual perception of objects by birds. Animal behaviorists and visual ecologists have developed visual models (i.e., mathematical algorithms that mimic some mechanisms of visual processing in the avian retina) to estimate quantitatively how different species of birds see their worlds. I will explain the potential of applying these visual models to poultry species in terms of modifying their environment (color of objects, light spectral property and intensity) to improve welfare and ultimately enhance production.

Key Words: vision, welfare, visual perception

498S Water efficiency and sustainability in broiler production: A corporate perspective. Jamie Burr*; *Sustainability, Tyson Foods Inc, Springdale, Arkansas, United States.*

As one of the world's largest food companies, Tyson Foods has the responsibility to help build a more sustainable food system. We have set bold goals and commitments that aspire to sustainably feed our growing world. Our number one priority is to provide safe food. We consider protein to be the "hero nutrient," playing an essential role in a well-balanced diet. Consumers' interest in their overall health is driving food choices in the marketplace. Throughout our value chain, we keep animal welfare top of mind and urge our independent farmer partners to do the same. On the farm, company veterinarians and animal welfare specialists work closely with farmers to ensure optimal animal husbandry, nutrition, sanitation and housing practices that support animal health and well-being. Tyson depends on land, water, and energy to grow the ingredients, raise the animals, and run our facilities to produce our food products. Accordingly, environmental stewardship is a core value of Tyson Foods' business philosophy and commitment to sustainability. We believe protecting the environment and conserving natural resources is essential for maintaining clean air, water, and land. Water touches everything we do at Tyson Foods - from the irrigation needed to grow the grain that feeds poultry and livestock to our processing plants where we use water to process animals, cook prepared foods and clean our facilities. Approximately 31 billion gallons of water enter our facilities annually. Tyson Foods is committed to bold reduction of our carbon footprint. We are working toward a "30 by 30" target to reduce greenhouse gas (GHG) emissions 30% by 2030 against a 2016 baseline year. This target is designed to meet the criteria of the Science Based Targets initiative (SBTi), which accepted our target in 2018, making us the first U.S. protein company in the food and beverage sector to receive

such an approval. In summary, Tyson's purpose is to raise the world's expectations for the good food can do. Sustainability spans several pillars as indicated by the five focus areas. As a leader in protein production across the world, Tyson takes our responsibility seriously.

Key Words: Sustainability, water efficiency, environmental stewardship

499S Water conservation and preservation of broiler production using sprinkler technology in hot environments. Tom Tabler*¹, J. Moon¹, J. DuBien¹, Y. Liang², Sami Dridi²; ¹*Mississippi State University, Mississippi State, Mississippi, United States*, ²*University of Arkansas, Fayetteville, Arkansas, United States.*

Sustainability and water conservation are critical to a poultry industry faced with global production challenges that include an increased demand for high-quality, affordable animal protein and greater environmental pressures resulting from climate change, heat stress, and limits on water availability. For two summer flocks (May-July and Aug-Oct 2020), a commercial sprinkler system used in combination with a cool cell system (SSCC) was evaluated against a cool cell only system (CC) in two commercial broiler houses (42 x 400 ft; 13 x 122 m) at Mississippi State University to determine effects of sprinkler technology on cooling water conservation, preservation of broiler performance, and in-house broiler environments. Two lines of overhead sprinklers 10 ft off the N and S sidewalls spaced evenly 20 ft (6 m) apart and 7ft (2.1 m) above the litter surface intermittently applied controlled volumes of water droplets onto birds. Hs 1 was SSCC house during the first flock. Cooling arrangements were reversed on the second flock, with Hs 2 being SSCC. Environmental data (temperature, humidity, and litter moisture) were collected periodically throughout both flocks. Production data (feed conversion ratio (FCR), body weight, daily mortality, paw quality, water consumption, cooling water usage) were calculated and recorded throughout the flocks. Data were analyzed as a Randomized Complete Block Design using SAS 9.4. Because there was no house effect ($P > 0.05$), treatments were compared across flocks. With higher average temperatures in SSCC than in CC of 3°F ($P = 0.08$), average water usage by SSCC was 64% less than CC, and average humidity across flocks was lower in SSCC than in CC ($P = 0.054$). Litter moisture for SSCC tended to be lower but showed no difference at several time points between and across flocks ($P = 0.11-0.16$). There were no differences in any of the other measurements. Final body weight was 0.1 lb higher and average FCR was 0.04 lower in SSCC, neither being significant; however, rate of return was 0.155 cents per pound higher ($P = 0.02$) on birds from SSCC. Although replication was an issue in this study, as is always the case with whole-house treatments, results are similar to those we've reported previously and provide additional evidence that sprinklers in conjunction with cool cells preserve broiler production and conserve cooling water, thereby improving sustainability. Future research

should investigate optimum flock age and house temperature to begin sprinkler and CC cooling.

Key Words: Water conservation, Sustainability, Humidity, Sprinkler, Broiler cooling

500S Commonalities, differences, and control measures of common causes of enteric inflammatory insults in commercial poultry and impacts on nutrient absorption and water conservation. Billy M. Hargis, Walter G. Bottje, Guillermo Tellez-Isaias*; *Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States.*

Moderate to severe enteric inflammation has long been known to interfere with nutrient and water absorption (malabsorption), with severe inflammatory gut disease of poultry commonly associated with the passage of macroscopically-obvious undigested feed (feed passage). Lesser chronic conditions of inflammatory enteric disease in monogastric animals have been associated with vitamin and mineral absorption deficiencies as well as systemic electrolyte imbalance and contribute to subclinical but economically important malabsorption. Even psychogenic or physical stressors for poultry cause rapid onset of mild to moderate malabsorption associated with reduced tight gap function within the epithelium (leaky gut). Immediate transitions to alternative feedstuffs, particularly those containing high levels of non-starch polysaccharides (NSPs) and some mycotoxins, are frequently associated with dysbiosis which is often more than transitory. Enteric inflammatory responses to dysbiosis and associated toxin production produces a secretory diarrhea, as do opportunistic Gram negative pathogens which have overgrown. Notably, there are a large number of enteric viruses for most vertebrate species which, although nearly ubiquitously present, are seldom diagnosed. Often viruses with enteric tropism present clinically as intermittent diarrhea (flushing) and are associated with various degrees of selective malabsorption. Importantly, a number of protozoal pathogens, including the nearly ubiquitous *Eimeria* genus in poultry cause subclinical to clinical coccidiosis. Coccidiosis (and other protozoa) are known to cause rapid and profound inflammation and inflammatory leukocyte infiltration of the enteric lamina propria. *Eimeria* species can cause acute and repeated destruction of the epithelial absorptive surface and perhaps, in aggregate, contribute to the greatest infectious cause of malabsorption for poultry species, with infections with some species predisposing to important opportunistic pathogens such as *Clostridium perfringens*, the causative agent of necrotic enteritis. Moreover, several *Eimeria* species and other protozoa (e.g. *Eimeria tenella* in chickens) have tropism for the ceca and are capable of causing severe typhilitis, thereby interfering with water reabsorption from retrograde transport of the dilute urine from the cloaca of poultry, causing incredible water loss (and wet litter) of poultry. Each of the above causes of enteric inflammation, epithelial cell destruction, and leaky gut can greatly impact feed efficiency, litter moisture, and water conservation in

commercial poultry. These impacts, mechanisms of action, and control measures will be reviewed in this seminar.

Key Words: Enteric inflammation, *Eimeria*, *Clostridium*, leaky gut, water efficiency

501S Divergent selection for water efficiency in commercial broilers. Joseph Hiltz, Nicholas Anthony, Sara Orłowski*; *Poultry Science, University of Arkansas, Springdale, Arkansas, United States.*

Freshwater scarcity is a growing global concern as urbanization and inadequate supply infrastructure challenges general sustainability. The anticipated population growth will further strain freshwater resources to meet both human needs and agriculture applications. We must do our part to ensure a water sustainable and food secure future by reviewing all aspects of agriculture where water usage can be managed and made more efficient. The challenge with water management is often tied to the inability to accurately measure and distinguish between consumption and waste. For the current study the potential of improving water efficiency in broilers is explored. The first strategy was to develop more efficient and accurate methodologies for measuring water consumption/inputs in agriculture. To date, water measuring technology has lacked the necessary sophistication to assure accuracy and repeatability of low flow water usage. After the establishment of a low flow water monitoring system, it was used to develop water efficient and inefficient broiler lines. This was done too not only determine heritability of water efficiency but to assess the response to selection and impact on correlated traits after several generations of selection. From a modern random bred population, lines were divergently selected based on water conversion ratio (WCR = water consumed/body weight gain) to create the low WCR (LWCR) and high WCR (HWCR) lines. After generation 2, the LWCR line had an overall WCR of 3.28 while the HWCR had a WCR of 3.46. Body weights appeared to remain similar between the lines with a slight improvement in feed conversion ratio observed in the LWCR line. Continued selection for WCR provide further understanding in the heritability of the trait and the response to selection of growth and efficiency related traits.

Key Words: water conversion, water scarcity, broilers, divergent selection, genetics

502S Improving phytase accumulation in *Chlamydomonas reinhardtii* for animal feed applications by modeling RNA folding. Michele G. Croen¹, Jennifer A. Schmidt¹, Terry W. Campbell¹, Mohammad Yazdani¹, Xin Gen Lei², Beth A. Ahner*¹; ¹*Biological and Environmental Engineering, Cornell University, Ithaca, New York, United States,* ²*Department of Animal Science, Cornell University, Ithaca, New York, United States.*

Single celled eukaryotic algae are being engineered to simultaneously produce high-value proteins for animal feed

to augment the commercial value of algae-based feed which are naturally high in important nutritive pigments and unsaturated fatty acids. We are using a chloroplast genome insert to engineer accumulation of phytase in *Chlamydomonas reinhardtii*, a model algae species. Because chloroplast-engineered *C. reinhardtii* do not reliably accumulate foreign protein and successful yields of 5% of total soluble protein (TSP) are rare, we are working on tools to improve gene cassette design. Using *in silico* RNA folding models, we compared the folding patterns of published successful and unsuccessful transgenic cassettes and found that success is correlated with constructs that maintain the native folding pattern of the 5' UTR and do not generate strong base pairing with the start codon. We have designed two new downstream boxes (DBs), or short coding regions just following the start codon, to improve the translation of *appA*, a phytase from *Escherichia coli* that has been modified for increased heat stability and codon optimized for expression in the chloroplast. We have successfully measured phytase expression for one of the new cassettes and are continuing work to generate two additional strains which we will use to confirm our hypothesis about the importance of mRNA folding for foreign protein accumulation in algal chloroplasts.

Key Words: microalgae, phytase, mRNA folding

503S Impact of diet on water use efficiency in broiler production. Samuel J. Rochell*¹, Michael T. Kidd¹, Xin Gen Lei²; ¹*University of Arkansas, Fayetteville, Arkansas, United States*, ²*Department of Animal Science, Cornell University, Ithaca, New York, United States*.

Efficient water use at the farm and individual bird level is critical to the welfare and sustainable production of commercial poultry. Drinking behavior and water utilization by broilers are influenced by several factors such as diet composition and feeding program. In particular, dietary mineral balance, protein content, and carbohydrate composition have all been demonstrated to markedly influence water consumption and/or excretion by broilers. Subsequent effects on nutrient digestion and absorption arise from altered intestinal pH, osmolarity, and digesta viscosity and rate of passage. Such changes to the intestinal environment can also influence litter quality and susceptibility to enteric pathogens, both of which are key determinants of bird welfare. To this end, the relationship between dietary nutrient composition and water use efficiency will be addressed, with emphasis placed on current practices and emerging innovations in broiler nutrition.

Key Words: nutrition, water efficiency, osmoregulation, flushing, wet litter

504S Impacts of supplemental dietary microalgae on water intakes of broiler chickens. Xin Gen Lei*; *Department of Animal Science, Cornell University, Ithaca, New York, United States*.

Defatted microalgae from biofuel production contain high concentrations of protein with a good balance of amino acids and other bioactive nutrients. Previous studies have demonstrated benefits of feeding broiler chickens with such biomass to their growth performance, health status, and meat values. Although water is an important nutrient and water use for animal production is becoming an increasing concern, past research has overlooked impacts of supplemental dietary microalgae on water intake or water use efficiency of broilers. Our laboratory fed day-old Ross chicks (total = 180) a corn-soybean meal basal diet containing 0, 2, 4, 8, or 16% of defatted *Nannochloropsis oceanica* (crude protein = 38.2%, Cellana, Kailua-Kona, HI) (6 cages/group and 6 chicks/cage) for 3 wk. All birds had free access to feed and water. Water was provided in 500-mL chick waterers for wk 1, and then in 3 L water pans for wk 2 and 3. Water intakes were recorded daily. Birds had a linear increase in water intake in response to the increased microalgae inclusions at wk 1 ($P < 0.01$, $R^2 = 0.29$), 2 ($P < 0.0001$, $R^2 = 0.82$), and 3 ($P < 0.0001$, $R^2 = 0.91$). The linear increase remained significant ($P < 0.0001$, $R^2 = 0.76$) for the total water intakes of birds during the 3-week period. Chicks fed the 8 and 16% microalgae diets over this period consumed 16 to 39% ($P < 0.05$) more water than those fed the control diet. Although the 8% microalgae diet did not depress growth performance, the extra water usage will lead to not only higher demand for the agricultural water needs but also a larger amount of litter. A high salt concentration (4.7% Na) in the marine green microalgae was the apparent reason for the elevated water intakes of birds. Subsequent analysis found heavier heart and/or liver weights in those fed 8 or 16% microalgae than the control birds, indicating an extra metabolic load. In conclusion, additional processing is needed to remove the extra salt present in the marine microalgal biomass for the full nutritional values without adverse metabolic and environmental impacts. (Supported by DOE/USDA Biomass Research and Development Initiative grant 201110006-30361, DOE MAGIC grant DE-EE0007091, USDA grant 2019-69012-29905, and Cornell University Hatch grants NYC-127302).

Key Words: broilers, environment, microalgae, water intake, sustainability

505S Intestinal nutrition during challenge scenarios: knowledge and gaps regarding the role of amino acids. Samuel J. Rochell*; *University of Arkansas, Fayetteville, Arkansas, United States*.

Amino acids (AA) are among the most economically important nutrients in poultry diets. Indeed, the performance and meat yield of modern broiler strains have proven to be responsive to increased dietary AA density, driving trends toward higher AA concentrations in broiler feed formulations. Before amino acids can become available for skeletal muscle synthesis in the periphery, they must escape first-pass intestinal metabolism. A large proportion of AA are catabolized by intestinal enterocytes as energy substrates and are also incorporated into mucosal proteins. This

already high intestinal demand for AA is heightened under gastrointestinal stress arising from environmental or pathogenic challenges. Concurrently, protein digestion and AA absorption are often compromised in response to these stressors, leading to increased undigested protein in the distal small intestine and hindgut. This alone can have consequences on the luminal environment that generally favor enteric pathogens and increase intestinal stress. Therefore, opportunities remain for a better understanding of how the modern broiler's robust needs for digestible AA, including those of the intestine, can be more precisely met without over-supplying indigestible protein. Existing knowledge and current gaps in our understanding of the dynamics of enteric AA metabolism for both healthy and challenged broilers will be discussed.

Key Words: amino acid, intestine, enteric challenge, precision nutrition, broiler

506S Intestinal nutrition: role of vitamins and biofactors and gaps of knowledge. Douglas R. Korver*; *Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, Alberta, Canada.*

The role of the microbiota in the health of the host is complex and multifactorial. The microbiota both consumes nutrients in competition with the host, but also creates nutrients that can be used by the host. However, the quantitative impact of the microbiota on nutrient supply and demand is not well understood. The costs and benefits of the microbiota on the nutrient economy of the host will be discussed, and knowledge gaps will be identified as

potential areas for future research, with a special emphasis on vitamins and biofactors.

Key Words: chicken, microbiota, vitamin, biofactors, nutrition

507S Role of diet-microbiota interactions in precision nutrition of the chicken. Michael H. Kogut*; *USDA-ARS, College Station, Texas, United States.*

Although many host-derived factors are hardwired and difficult to modulate, the microbiome may be more readily reshaped by dietary exposure and is increasingly recognized to potentially impact poultry physiology by participating in digestion, the absorption of nutrients, shaping of the mucosal immune response, energy homeostasis, and the synthesis or modulation of a plethora of potentially bioactive compounds. These activities are dependent on the quantity and quality of the microbiota alongside its metabolic potential, which are dictated in large part by diet. Thus, diet-induced microbiota alterations may be harnessed to induce changes in host physiology, including disease development and progression. In this regard, the gut microbiome is malleable and renders the gut microbiome a candidate 'organ' for the possibility of precision microbiomics—the use of the gut microbiome as a biomarker to predict responsiveness to specific dietary constituents to generate precision diets and interventions for optimal poultry performance and health.

Key Words: Gut microbiome, nutritional immunity, biotics, metabolites, intestinal physiology

Latin American Scientific Conference Abstracts

Note: These abstracts were submitted for presentation at the 2021 PSA Latin American Scientific Conference. However, when the conference was postponed due to COVID-19, the authors were invited to present their abstract as part of the 2021 PSA Annual Meeting.

508L How do broiler farmers picture broiler welfare and perceive technologies potentialities on welfare improvements? Heitor Rios*^{GS}, Paulo D. Waquil; *CEPAN, UFRGS, Porto Alegre, Brazil.*

A questionnaire regarding broiler welfare and technologies aiming at animal welfare improvements were applied to 184 broiler farmers of Southern Brazil. The objectives of this study were two-fold: (i) to analyze the opinions of farmers on possible broiler welfare improvements and (ii) to analyze their opinions on technology potentialities to improve broiler welfare. The criteria and principles present in Welfare Quality® (WQ®) protocol for broiler chickens were used to guide questions. Broiler farmers were asked to score the importance welfare has to them and their perceptions regarding the current conditions of living of broilers for each WQ® welfare criteria. Higher scores for importance than to perceptions indicate that criteria is passive of improvements. The opinions of farmers on technology potentialities were evaluated by analyzing their level of agreement in a five-point Likert scale to four statements regarding the principles of broiler welfare. Data were submitted to Mann Whitney/Kruskal Wallis tests at 5% significance and to Dunn test with p-value adjusted for Bonferroni. Spearman correlations were performed to analyze how the opinions on broilers welfare correlates to the opinions on technology potentialities. Broiler farmers scored higher values for the importance of the criteria within good feeding and good health principles than for their perceptions for the same criteria ($P < 0.05$). Regarding the good housing principle, broiler farmers attributed higher scores for the importance of “comfort around resting” and “thermal comfort” than to their perceptions ($P < 0.05$); however, no difference was observed for the criterion “ease of movement” ($P > 0.05$). No differences were observed between importance and perception for criteria withing appropriate behavior ($P > 0.05$). Broiler farmers scored higher values for technology potentialities to improve good feeding and good housing when compared to good health and appropriate behavior ($P < 0.05$). A positive correlation between the importance attributed to broiler welfare and opinions on technology potentialities were observed for good feeding, good housing and appropriate behavior ($P < 0.05$). The perception scores attributed by broiler farmers to good housing and appropriate behavior were positively correlated to their opinions on technology potentialities to improve such principles ($P < 0.05$). In general, broiler farmers indicated that there would be room for welfare improvements except for the criteria within appropriate behavior and for “ease of movement”. Farmers

indicated that technologies could assist them on broiler welfare improvements, especially regarding the good feeding and the good housing principles.

Key Words: Poultry welfare, Information technology, Welfare assessment, Farmer perception, Welfare quality

509L Identification of reliable reference genes for expression studies in digestive system tissues of laying hens housed in cage and cage-free systems. Karen D. Diaz Solano*¹, Roy Rodríguez Hernández², Iang S. Rondon Barragan²; ¹Biology, University of Tolima, Ibague, Tolima, Colombia, ²Animal Health, University of Tolima, Ibague, Tolima, Colombia.

Stress and dietary factors during egg production can produce changes in gene transcription and protein synthesis in the hen digestive system and could affect the digestion, secretion of mucin and absorption of nutrients. The objective of the study is to identify the most stable reference genes for data normalization in gene expression studies in proventriculus and duodenum tissues of hens housed under two egg production systems. Under commercial conditions, approximately 60,000 Hy-Line Brown pullets were reared with the same conditions until 15 weeks of age. Later, they were transferred into two different production systems, conventional cage (CC) and cage free (CF), on the same farm, up to 82 weeks of age. At 82 weeks, a total of three (3) hens from CC and three (3) hens CF systems were randomly selected from different replicates and euthanized by cervical dislocation followed immediately decapitation. Approximately 0.5 g of proventriculus and duodenum tissues were collected and immediately stored in RNAlater® stabilization solution, and frozen at -20°C until analysis. RNA extraction was performed using RNA-Solv® reagent and cDNA synthesis using a high throughput cDNA reverse transcription kit. β -actin (actb), 18S rRNA (18s), glyceraldehyde-3-phosphate dehydrogenase (gapdh), Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (yw haz) and hydroxymethylbilane synthase (hmbs) genes were selected for this study. qPCR was performed using QuantStudio™ 3, by Fast ramp speed program. Each sample was run in duplicate. Data analysis was conducted using four software: geNorm, NormFinder, BestKeeper and RefFinder, for the identification of reliable reference genes. The results showed the most stable genes in proventriculus tissues of hens in CC and CF using geNorm were: 1. yw haz, 2. gapdh 3. hmbs. NormFinder: 1. gapdh, 2. yw haz, 3. Actb. BestKeeper: 1. yw haz, 2. actb, 3. 18S. RefFinder: 1. gapdh, 2. yw haz, 3. actb. In duodenum tissues were in geNorm: 1. gapdh, 2. hmbs, 3. yw haz. NormFinder: 1. gapdh, 2. yw haz, 3. hmbs. BestKeeper: 1. yw haz, 2. 18S, 3. Gapdh. RefFinder: 1. gapdh, 2. yw haz, 3. 18S. In summary, gapdh, yw haz and actb genes were the most stable genes in proventriculus tissue, and gapdh, yw haz and hmbs genes in duodenum tissue of hens in CC and CF. The use of these reference genes for the normalization of

gene expression data may allow greater reliability of data analysis.

Key Words: welfare, qPCR, reference genes, proventriculus, duodenum

510L First genetic characterization of Infectious Bursal Disease Virus isolated from Chile. Miguel Guzmán^{*1, 2}, Leandro Cádiz², Leonardo Sáenz³, Héctor Hidalgo²; ¹Universidad de Las Américas, Santiago, Chile, ²Laboratory of Avian Pathology, Universidad de Chile, Santiago, Chile, ³Laboratory of Veterinary Vaccines, Universidad de Chile, Santiago, Chile.

Hypothesis or objective : Infectious Bursal Disease (IBD), is a highly contagious infection affecting young chickens worldwide, caused by the Infectious Bursal Disease Virus (IBDV). IBDV is a non-enveloped virus highly resistant to environmental conditions, resulting in high prevalent disease. In South America has been described genogroups 1 (G-1), 3 (G-3), and 4 (G-4). The aim of the present study is to present a detailed genetic characterization of IBDV obtained from Chilean poultry commercial flocks, it would be expected that Chilean Viruses would have similar diversity to other countries from the region. Experimental design (or description of the problem for a non-statistical presentation): IBDV has been isolated in Chile, however, there is not any paper describing its genetic characteristic, nor classifying these viruses into the pre-established genogroups. Materials and methods: Ten chickens between two and six weeks old from different commercial poultry flocks were received at the Avian Pathology Laboratory, then the Bursae of Fabricius removed. Viral extraction, cDNA production, PCR, and PCR product purification were performed using commercial kits (Invitrogen) following the manufacturer's guidelines. A 604 bp amplicon was produced with U3 – L3 primers previously published by OIE. Finally, they were sequenced at MacroGen Inc. Consensus sequences were assembled with Bioedit v 7.2.5, and aligned using the software Mafft v.7.2. PhyML 3.0 platform was used to construct the phylogenetic tree, performing 1,000 bootstraps to support the robustness of the nodes, considering representative sequences from all the Genogroups. MEGA X was used to estimate evolutionary divergence in the VP2 hypervariable region. Results: Chilean sequences of IBDV were clustered in a unique well-defined clade within the G-1. No other genogroup was associated with the viruses obtained in this study. Moreover, the amino acid distance between Chilean sequences and G-1 was calculated at 0.0492, while the distance with the others genogroups ranged between 0.0831 (G-3) and 0.1051 (G-5). Comparing amino acid residues, only positions 223 and 279 showed differences with ViBursaCE and D78 vaccines, which are currently used in Chile. These positions have shown to be involved with antigenic properties in VP2 and the ability to replicate in chicken embryonic fibroblasts, respectively. Conclusions: This is the first genetic approach to understanding the IBDV circulating in Chile. No variant strains appear to be involved in field cases, all of the viruses

belong to G-1. G-3 and G-4 which have been reported in the rest of South America were absent in Chilean IBD cases.

Key Words: Infectious Bursal Disease, Phylogeny, Genogroups, Maximum likelihood

511L Gut health markers of the efficacy of *Bacillus subtilis* DSM 29784 in broiler chickens. Damien Preveraud^{*1}, Tim Goossens², Estelle Devillard³; ¹Health by Nutrition, Adisseo France SAS, Antony, France, ²Health by Nutrition, Adisseo, Dendermonde, Belgium, ³Center of Excellence and Research in Nutrition, Adisseo, Commeny, France.

Biomarkers to evaluate gut health are difficult to determine with precise accuracy but they are necessary to predict animal performance and to evaluate efficacy of gut health solutions. The aim of this work is to determine the effect of the probiotic solution *Bacillus subtilis* DSM 29784 (Bs29784) on the most relevant gut health biomarkers in poultry production. To that manner, we have summarized the data obtained in different in vivo models. The first approach was to do the macroscopical scoring of the intestine. The method described in Teirlynck et al (2011) showed negative correlation with villus length and positive correlation with T-cell infiltration in dysbacteriosis condition. In our trial, a dietary challenge was induced by increasing NSP content. The Bacterial Enteritis Score was 2.0 for the control group and decreased to 1.0 with the supplementation of Bs29784. Then, a microscopical evaluation of the gut gives a precise state of the epithelial integrity and inflammation. For instance, longer villi height is associated with higher body weight. On 42 day-old broilers, Bs29784 significantly increased villi length relative to control from 0.66 to 0.79 μm in the ileum, and from 1.25 to 1.46 μm in the cecum. Looking at the microbiota from an ecological perspective could provide good insight as well as it is assumed to be as resilient as possible if the population is diverse and contains more bacteria with anti-inflammatory properties (butyrate producers like Ruminococcaceae and Lachnospiraceae) and less opportunistic pathogens like *Clostridium perfringens* or Enterobacteriaceae. In a necrotic enteritis challenge model, Bs29784 group showed higher alpha-diversity than control birds with Chao and Shannon index. We also observed higher prevalence of Ruminococcaceae with the supplementation of Bs29784, especially for *Faecalibacterium prausnitzii* (+13.4%) that is known to be correlated with villus length and CD3 area. In another trial where broilers were challenged with *C. perfringens*, we observed that the prevalence of *E. coli* at 21 d and *C. perfringens* at 28 d were significantly reduced in the Bs29784 group. Finally, some microbial metabolites can be linked to gut health. Nicotinic acid for instance activates the GPR109A receptor, which suppresses intestinal inflammation. We demonstrated that it increases cell proliferation and wound healing in a Caco-2 in vitro model. An exploratory study on 13 day-old chicks showed that Bs29784 increased the intestinal content of nicotinic acid in

ileum and jejunum, revealing a mode of action through which it can improve gut health. This work demonstrated that these key markers can be measured to evaluate the consistent efficacy of Bs29784 in broilers.

Key Words: p biomarkers, bacillus subtilis, microbiota, gut barrier, immunity poultry

512L Positive Impact of Gumboro control on broiler performance, uniformity and results in the slaughterhouse. Higor Cotta¹, Mathilde Lecoupeur¹, Thomas Lewiner¹, Jorge Chacon¹, José Maurício França², Marco Aurélio Elmer Lopes^{*1}; ¹*Ceva, Libourne, France*, ²*Universidade Tuiuti do Paraná, Curitiba, Brazil*.

Introduction: Gumboro disease is one of the most important diseases for the poultry industry, infecting the bursa and impacting the immune system and the general health of the broilers. As known, it can impact the results in the field and the processing of the broiler's carcass in the slaughterhouse. When the subclinical form of Gumboro disease is present due to a vaccination failure, which can be verified by looking at the flock's serology titers, the chickens are infected by the field virus. Objective: In this context, this paper aims to assess the impact on field and slaughterhouse performance of the subclinical Gumboro by contrasting two different scenarios of disease pressure. Experimental Design: Farm and slaughterhouse serology and performance results from a South American broiler producer are presented. Thirty mixed broilers flocks from 21 different farms in a total of 1,419,200 birds divided into two groups vaccinated in-ovo: G1 by the reference IBD immune-complex vaccine (TRANSMUNE®) G2 by others hatchery IBD vaccines. Blood samples were collected at slaughter time (47 days of age (DOA)). For each flock, farm performance variables are the average of DOA, body weight (BW), feed conversion rate (FCR), average daily gain (ADG), European production efficiency factor (EPEF), geometric mean titer (GMT), and total mortality as well the mortality after 35 DOA. The slaughterhouse performance variables are mortality during transportation, full and partial condemnations by airsacculitis, fecal condemnation and cachexia, and slaughter line downtime. Statistical significance was assessed by the Mann-Whitney test, and a p-value < 0.05 was deemed significant. Results and discussion: Contrasting G1 and G2 field results, most of the variables were significant. We highlight that all mortality variables were favorable to G1 whilst BW, ADG, and FCR were not significant. Considering the slaughterhouse performance variables, the significant one was the partial fecal condemnation. However, since it is known to impact slaughter line cadence speed (to reduce the contamination risk), improving this variable leads to better economic results. Although not achieving statistical difference, all results were favorable to G1. For instance, the total downtime in minutes G1 had 66.37 (52), mean (SD), and G2 109.18 (155), as a processing performance indicator, impacts on the product economic feasibility and the commercial profitability. In this direction, G1 had a GMT

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of 4,000 (1,613), while G2 had 5,090 (1,174). Flock's having GMT greater than 4,000 are well protected against the subclinical form and controlling Gumboro disease leads to better field and slaughterhouse performance results.

Key Words: Gumboro, subclinical, slaughterhouse, economics, performance

513L No presentation materials submitted.

514L No presentation materials submitted.

515L Effect of different source of yeasts on broiler performance. Ricardo V. Nunes^{*1}, Jomara Broch¹, Cristine Kaufmann¹, Nilton R. Junior¹, Thiago P. Ribeiro², Barbara Colcetta², Idiana Silva², Daniel Pigatto Monteiro², Guilherme Tesser¹, Nathanael Santos¹; ¹*Animal Science, Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon, Paraná, Brazil*, ²*Animal Nutrition, Tectron, Toledo, Brazil*.

The present study aimed to evaluate the effects of supplementation of different sources of yeasts on broiler performance. A total of 540 broilers Cobb 500 were allocated in cages from 1 to 42 days. Birds were distributed according to a completely randomized design with three treatments and nine replicates of twenty birds each. The treatments were: control treatment (T1 - without yeast); metabolic bioactive (MB) from yeast blend composed by brewer yeast, baker yeast and sugar cane yeast (T2 - 1 kg t⁻¹ of MB) and commercial blend yeast (T3 - 1 kg t⁻¹ of BY). All birds and feed remaining in the feeders were weighed by repetition, to determine the weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) at 21, 35 and 42 days. The normality and homogeneity of the data was determined by the Kruskal Wallis and Levene's test and for the removal of outliers, the Interquartil system (SAS program) was used. Subsequently, data were analysed by ANOVA, and the significant differences (P<0,05) values were compared by Tukey's teste. There was no difference for the performance at 21 and 42 days. However, at 35 days, birds fed T2 had improved FCR than other treatments. The efficiency of MB supplementation may be associated to several factors like source, diets, concentration and other else. This FCR improvement indicates that the supplementation of MB from yeast is due to the one beneficial effects of yeast in improvement of the intestinal lumen health and thereby increasing the absorption and utilization of the dietary nutrients. In the conditions evaluated, the use of metabolic bioactive from yeast blend composed by brewer yeast, baker yeast and sugar cane yeast benefited the feed conversion ratio of broilers at 35 days.

Key Words: additives, intestinal health, poultry nutrition, prebiotics

516L Effect of dietary balanced protein on body composition and the age at first egg of laying hens. Ingrid Nóbrega^{* GS 1}, Rony Lizana¹, Thaila Moura¹, Guilherme Teófilo¹, Raully Silva¹, Letícia Bittencourt²,

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The objective was to evaluate the variation on the body composition and their effect on the early sexual maturity of pullets fed with balanced dietary protein levels. 280 Lohman LSL pullets were used from 52 to 212 old-d. The birds were randomly assigned to one of the three treatments consisting of levels of balanced protein (BP): control (C), reduced (C-20), and increased (C+20) in 20% of the BP above or below control group. The lysine level of control was 0.803, 0.700, 0.742 and 0.680 for growth, development, pre-laying and laying phases, respectively. At the start and end of each phase were collected the following data: body weight (BW), and body composition (ash, lipid, protein, and water) by DXA scan, which was expressed relative to BW (g/100g of BW). Additionally, the egg production (EP) was recorded daily, and it was counted from the laying of the first egg of each treatment. The average age at first egg (AFEm) was considered to be 50% of EP. The body composition data were analyzed by ANOVA (BP×Age), and the mean comparison was by Tukey test. The EP for each treatment was fit to the Gompertz model modify: $EP=100*EXP(-EXP(-k*(Age-t)))$, where 100 is the asymptotic value, k is the rate and t is the inflection point. The AFEm was estimated from this function. It was not shown interaction for any of the evaluated variables. The body composition varied significantly ($P<0.05$) along with the age. On the other hand, the BP had a significant effect on BW ($P=0.0043$), the C+20 (1,175 kg) was higher than C (1,109 kg) and C-20 (1,107 kg); the opposite was shown for ash content ($P=0.0039$), where C+20 (3.86) was lower than C (4.02) and C-20 (4.00). Also, the lipid content was different ($P=0.0171$) between C (11.88) and C + 20 (12.78), but not different from C-20 (12.22). The body protein was not affected ($P=0.133$) by the BP levels with an average of 18g/100g. The parameters estimated for EP curve were lower to that parameter t for C+20 (13.11 ± 0.225), varying on around one day for C and four days for C-20; likewise, the rate was higher for this treatment (0.168 ± 0.0075), compared with C (0.14 ± 0.0042) and C-20 (0.121 ± 0.008). Based on that, the estimated AFEm were 16.9, 20.42 and 15.29 for C, C-20 and C+20; respectively. In conclusion, the BP modifies the body composition of pullets on the rearing phases, and affecting the early laying of birds, and consequently on the increasing of egg production along the productive cycle of the laying hens.

Key Words: Age at first egg, Animal nutrition, Balanced protein, Body composition, Laying pullets

517L No presentation materials submitted.

518L Improving feed efficiency by boosting digestive and exogenous enzymes activity thanks to clay and algae. Marie Gallissot^{*1}, Aleixo Pedro², Luiz Fernando T. Albino², Horacio S. Rostagno², Raquel Pereira¹, Maria Angeles Rodriguez¹; ¹*Olmix, Bréhan, France*, ²*Agricultural*

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Several types of feed additives are available to improve feed digestibility, including exogenous enzymes which target specific nutrients that are not digested by endogenous enzymes. Recently, an innovative algo- clay complex (ACC) developed by Olmix Group (France) has shown interest in increasing the activity of endogenous digestive enzymes and feed efficiency. The present work aims at reviewing the effect of this ACC on broilers performance depending on the use of exogenous enzymes in the diet. Three different studies were implemented in the Poultry Research Center of the University of Viçosa (Brazil). All studies used Cobb 500 male broilers fed corn- soybean diets. Animals were placed in pens of 20 (Study 1) or 22 (Studies 2 and 3) chicks, with 10 replicates per treatment. In each study, the treatments were control (no ACC supplementation) and test (with 0.1% ACC in all feeding phases). The diets in the 3 studies differed by their supplementation in exogenous enzymes: no exogenous enzymes were used in Study 1, phytase was used in all groups in Study 2 and phytase and carbohydrases (NSP enzymes) were used in all groups in Study 3. Results of the Study 1 showed a decrease in feed conversion ratio (FCR) when using the ACC in grower phase (day 22-35; -4%, $P<0.05$) and total cycle (day 0-35, -3%, $P<0.05$). Growth rate tended to be higher in the test group than control in the grower phase (+4%, $P<0.10$) with a final weight (at 35 days) of 2.449 kg in the test group and 2.392 kg in the control group ($P=0.127$). In the Study 2, the ACC supplementation lead to a decreased FCR of 2% in the total period (day 0-42, $P<0.05$) with the highest effect in the grower phase (-4%, $P<0.05$). The final weight (42 days) was increased by 78g in the test group compared to control (respectively 3.337 kg vs 3.259 kg, $P=0.163$). In the Study 3, the FCR (day 0-42) was decreased by 3% in the test group compared to control ($P<0.05$) with the highest effect in the finisher phase (-8%, $P<0.05$). Growth rate followed the same trend with an increase in average daily gain (ADG) of 4% ($P<0.01$), resulting in a 134g higher final weight (42 days). The highest effect was observed in the finisher phase with a 12% increase in ADG ($P<0.01$). All in all, these studies highlight the capacity of the ACC to improve feed efficiency and growth of broilers independently of the use of exogenous enzymes. The demonstration of a synergistic effect between the ACC and exogenous enzymes requires further investigation.

Key Words: Algae, Clay, Enzymes, Feed efficiency

519L Comparison of the use of glucose oxidase and BMD for the control of Enteropathogenic bacteria in broiler chicken. Noel Cesáreo^{*1}, Beatriz Ibarra Macari¹, Josue Sanchez Morales¹, Sergio Gomez Rosales², Maria de Lourdes²; ¹*Excelling, S.A. de C.V., Juriquilla, Queretaro, Mexico*, ²*Centro Nacional de Investigacion Disciplinaria en Fisiologia y Mejoramiento Animal – INIFAP, Ajuchitlan, Queretaro, Mexico.*

The long-term use of growth promoting antibiotics in

animal feed and their negative effects such as antimicrobial resistance and drug residues have set the tone for their replacement by non-antibiotic enzyme additives. Glucose Oxidase-GUTFITZYME3000 (GOX) is a flavoprotein that catalyzes the oxidation of β -D-glucose to D- glucono- δ -lactone and hydrogen peroxide using molecular oxygen as an electron acceptor, increasing the presence of beneficial bacteria that could help also as an important regulator to improve growth performance and intestinal health. The present work focused on verifying the efficacy of GOX and Bacitracin Methylene Disalicylate (BMD) for the control of coliforms and Salmonella spp. A group of 360 one-day-old male Ross 308 chickens was used. They were randomly distributed in 3 treatments with 8 replicates per treatment and 15 chicks per replicate. The farm has a house with a manually controlled natural environment. The pens have bell-type drinkers and hopper feeders. There were 3 production phases: initiation of 1-7 days, growth of 8-21 days and completion of 22-49 days. The preparation of the food was carried out in accordance with the recommendations of the lineage. In the period of 1-21 days, there were two treatments: treatment 1. food supplemented with 500 ppm of BMD, with 8 repetitions; Treatment 2. food supplemented with 200 ppm of GOX, with 16 repetitions. In the period of 22-49 days, there were three treatments: treatment 1. food supplemented with 500 ppm of BMD treatment 2. food supplemented with 200 ppm of GOX; treatment 3. food supplemented with 200 ppm of GOX. On days 1, 21 and 49, excreta samples were taken from a sample of chickens from each pen and a microbiological analysis was carried out to quantify the total count of bacteria, total coliforms and Salmonella spp. A completely randomized design with 8 repetitions per treatment was used. The response variables obtained were subjected to analysis of variance (SAS). Comparisons between means were made using the least significant difference method. The counts of total bacteria, total coliforms and Salmonella spp were statistically similar between the chickens supplemented with BMD and those that were added with GOX. The results indicate that it is possible to substitute the growth promoting antibiotic BMD for the GOX test product.

Key Words: broiler chicken, Bacitracin Methylene Disalicylate, glucose oxidase, Coliforms, Salmonella spp.

520L Effects of live *Saccharomyces cerevisiae* dietary supplementation on performance and immunity of broiler chickens. Alexandre B. Brito*, Gustavo Cordero, Gilson Gomes; *Technical, AB VISTA, São Paulo, São Paulo, Brazil.*

Probiotics, such as yeast *S. cerevisiae*, have been evaluated as potential feed supplement. The objective of this study was to evaluate the effects of a high concentration live yeast (*S. cerevisiae*, 20 billion CFU/g, Vistacell - AB Vista Marlborough/UK) on broiler performance, TGI development and immunity. 756 Cobb birds were used, distributed in 2 treatments (Control - without live yeast -;

and Live yeast-included at 250g/ton) with 14 repetitions of 27 birds/each. The birds were housed in a completely randomized arrangement, and the trial was carried out in a facility of a Indian Center, during the summer period (temp.C/hum.% varying from max 41/86 to min 23/36). Diets were corn and soybean meal based, offered as mash, with feeding regimen comprised of three feeding phases (Sta 1-14 days - 3,050 kcal of AMEn/kg and 1.3% of dLys; Gro 15-28 days - 3,100 kcal of AMEn/kg and 1.1% of dLys; and Fin 29-42 days - 3,150 kcal of AMEn/kg and 1.0% of dLys). Performance (body weight gain-BWG; feed intake-FI; feed conversion ratio corrected for mortality-FCR; body weight corrected FCR-bwcFCR and EPEF) was measured weekly, additionally, at 42 days of age the relative weight of the gizzard, cecum and intestinal tract were calculated. On days 28 and 42, blood samples were taken for determination of the Newcastle Disease Titers (NCT). Data was submitted to ANOVA and differences deemed significant at $P \leq 0.05$, with trends discussed when $P < 0.10$. Supplementation of live yeast significantly improved FI by 4g from 0 to 7 days of age (119 vs.123 g/b for Control and Yeast Group, respectively, $P=0.016$). From 29 to 35d BWG was improved on birds fed diets supplemented with live yeast by 32g (607 vs. 639 g for control and yeast, respectively, $P=0.013$). For the overall period (0 to 42 days of age) live yeast supplementation tended to increase BWG by 45g (2,250 vs 2,295 g/bird for control and yeast group, respectively; $P=0.08$), significantly improved FCR by 2 points (1.57 vs 1.55 g:g for control and yeast group, respectively; $P < 0.01$) and bwcFCR by 3 points (1.57 vs 1.55 g:g for control and yeast group, respectively; $P=0.01$). These culminated with a significant increase on EPEF by 11 points (306 vs 318 for control and yeast group, respectively; $P=0.039$). Exist a significant improvement in the cecum relative weight (0.42 vs 0.50% for control and yeast group, respectively; $P=0.022$), which may indicate an increase in fermentation capacity. As for NCT, there was a significant reduction in titers of blood sampled at 28d (3.4 vs 4.6 log for control and yeast group, respectively; $P < 0.01$). In conclusion, live yeast supplementation improved performance and increased the relative weight of the caecum, representing a potential probiotic for broilers.

Key Words: Live yeast, Performance, Cecum relative weight, Newcastle disease titers, Fermentation

521L Uncoated-buffered sodium butyrate addition improved performance and intestinal health of broilers fed low energy-protein diets. Julián E. Melo*¹, Florencia Prosdócimo¹, Federico Quintero¹, Ernesto Vignoni¹, Nélica Sosa¹, Matías Biondi¹, Mariano Batallé¹, Silvina Pinto², Xavier Roulleau³, Hebe Barrios⁴; ¹*Departamento de Tecnología, Universidad Nacional de Luján, Luján, Buenos Aires, Argentina,* ²*Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Buenos Aires, Caba, Argentina,* ³*Dietaxion SAS, Le Loroux Bottereau, France,* ⁴*Departamento de Ciencias Básicas, Universidad Nacional de Luján, Luján, Argentina.*

The objective was to evaluate the effect of an uncoated-buffered sodium butyrate (SB) supplementation on the performance, the jejunum histomorphometry and the duodenum coliform and lactic acid bacteria populations of broilers from 0 to 40 d of age when dietary energy and amino acids concentrations were reduced. A total of 360 1-d-old male chicks (Cobb-500) were allocated in 24 floor pens in a CRBD with 3 treatments (8 replicates/treatment). Pre-starter (0-14 d), starter (15-28 d) and grower (29-40 d) diets were used for dietary treatments. Control diets (CTL) were formulated according to Cobb recommendations (2018). Low energy-protein diets (LEP) were formulated to obtain a 3% reduction in ME, CP, and the main digestible amino acids (Lys, M+C, Tre). Another set of LEP diets were formulated to include SB (LEP+SB). SB (Butylin 54, Dietaxion) was included on-top during the whole experiment at 600 ppm. Birds and feed were weighed weekly individually and by pen, respectively, and the mortality recorded daily. At 14 and 28 days of age, one bird per pen was randomly selected and euthanized. The content of the last part of duodenum was used to examine microbial population (total coliforms and lactic acid bacteria were analyzed as log₁₀ cfu/g). Histomorphological examination of villus height (VH) and crypt depth (CD) of each animal was the mean of 5 determinations. Parametric data were analyzed as a 2-way ANOVA and non-parametric data through the Kruskal-Wallis test. There were not significant differences in body weight, colony counts of total coliforms and of lactic acid bacteria at any time ($P > 0.05$). CTL broilers had the lowest feed intake and FCR ($P < 0.05$) between 0-40 days of age, but also LEP+SB broilers had a lower feed intake and FCR than LEP ones ($P < 0.05$). LEP+SB birds showed a lower mortality ($P < 0.05$) than birds with the other diets during the first two weeks, but there were no differences at any other time ($P > 0.05$). Histomorphological traits VH, CD and VH/CD (VCR) were different between treatments ($P < 0.05$) at any time with the exception on VH at 14 days ($P > 0.05$). At 14 days LEP+SD broilers showed higher VCR and lower CD than CTL birds ($P < 0.05$) and at 28 days supplemented animals obtained a higher VCR and lower CD than the un-supplemented treatments ($P < 0.05$). At the last age LEP+SB broilers had a higher VH than LEP ones ($P < 0.05$). Dietary supplementation of broilers from 0 to 40 days with an uncoated-buffered SB improve the development of the upper part of the GIT increasing intestinal health of the jejunum and the feed efficiency in LEP diets. The use of SB in broilers fed LEP diets can be considered an important strategy to feed cost savings and excretion reduction.

Key Words: broiler, feed efficiency, intestinal histomorphology, sodium butyrate

522L Effects of calcium pidolate supplementation on performance, Ca-P retention, and bone development of broilers fed low Ca-P diets. Guillermo Fondevila Lobera^{2,3}, Nereida Luna Corrales^{2,3}, Julián E. Melo^{*1}, Xavier Roulleau⁴, Gonzalo González Mateos²; ¹Departamento de Tecnología, Universidad Nacional de Luján, Luján, Buenos Poul. Sci. 100 (E-Suppl 1)

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The effects of calcium pidolate (PID) supplementation on growth performance, calcium (Ca) and phosphorus (P) retention, and bone traits was studied in broilers fed low Ca-P diets from 0 to 21 d of age. The experimental design was completely randomized with 8 diets arranged as a 4 × 2 factorial with 4 levels of total Ca and P and the supplementation or not of 300 mg/kg of PID (PIDOLin PCa, Dietaxion SAS). Feeding periods were 0-14 and 15-21 d of age. The control diet contained 0.88% Ca and 0.63% P from 0-14 d and 0.73% Ca and 0.56% P from 15-21 d of age. The Ca and P levels of the experimental diets were 90, 85 and 80% of those of the control diets in each of the two feeding periods. Each treatment was replicated 10 times in cages with 10 Ross 308 birds each. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated by replicate from 0-7 d, 8-14 d, and 15-21 d of age. In addition, total tract apparent retention (TTAR) of dry matter, organic matter, Ca, and P of the diets was determined at 14 d of age. At 21 d of age, the weight, breaking strength, and elasticity of the tibia were determined in one bird per cage. Data were analyzed as a 2-way ANOVA and the linear (L) and quadratic (Q) effects of the Ca and P levels of the diet on all traits were studied in the two set of PID treatments (with or without PID, separately). There were not significant differences in body weight, ADG, or ADFI at any time ($P > 0.05$). From 0 to 21 d of age, PID supplementation affected ($P < 0.05$, for the interaction) FCR response of the birds to the levels of dietary Ca and P. A decrease in Ca and P contents impaired FCR in birds fed the unsupplemented PID diets (L, $P < 0.01$; Q, $P = 0.091$) but not in birds fed the supplemented diets (L, $P > 0.05$; Q, $P > 0.05$). Furthermore, the effect observed was greater in those groups of broilers fed the lowest Ca and P levels. The TTAR of P increased (L, $P < 0.01$) as the Ca and P contents of the diet decreased in broilers fed the PID diets but no effects were observed in birds fed the unsupplemented diets ($P > 0.05$). Dietary treatment did not significantly affect any of tibia variables measured ($P > 0.05$). Numerically, however, the breaking strength and elasticity of the tibia decreased by 5.5% and 4.8%, respectively, as the Ca and P content of the diet decreased in broilers fed the unsupplemented PID diets (L, $P = 0.086$) but not in broilers fed the supplemented diets. Supplementation with 300 mg PID/kg improved FCR and TTAR of P in broilers fed diets with low total Ca and P contents. The inclusion of PID in the diet for broilers fed low Ca and P levels could be considered an important strategy to reduce feed cost and mineral excretion.

Key Words: broiler, feed efficiency, calcium pidolate, P digestibility, tibia

523L Use of a blend of organic acid on broiler performance. Thiago P. Ribeiro^{*1}, Andreia Massuquetto¹,

Leandro da Silva¹, Daniel Pigatto Monteiro¹, Clauber Polese², Cleison de Souza², Andre Sanches de Avila², Felipe Potenza Campos², Ricardo V. Nunes²; ¹*Animal Nutrition, Tectron Innovation and Technology, Toledo, Brazil*, ²*Animal Nutrition, Universidade Estadual do Oeste do Paraná – UNIOESTE, Marechal Candido Rondon, Paraná, Brazil*.

This study aimed to evaluate the effects of the supplementation of a blend of organic acids (OA) on broilers performance. In total, 420 male broilers (Cobb 500) were housed in cages from 1 to 42 days of age. Birds were distributed according to a completely randomized design with three treatments and 7 replicates of 20 birds each. The treatments were: T1 - Negative Control (NC); T2 - NC + 1 kg t⁻¹ of OA (1 kg OA); T3 - NC + 2 kg t⁻¹ of OA (2 kg OA). The blend of OA was composed by benzoic acid, fumaric acid and citric acid. All birds and feed remaining in the feeders were weighed by repetition, to determine the weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) at 35 and 42 days. Means were compared by orthogonal contrasts. Each contrast was independently tested by the F test at 5% probability level. The birds that received 2 kg OA had lower FI at 35 and 42 days of age. At 35 days, the birds receiving the 1 kg O diet showed higher WG when compared to those fed 2 kg OA. And at 42 days, the birds that received 2 kg OA had better FCR than NC group. There are several publications explaining that organic acids have been used as an additive to improve the broiler performance significantly. Such a positive impact of dietary acidifiers on growth performance might be due to a reduction of pH values in the feed and digestive tract, killing the pathogenic organisms which are sensitive to low pH or selectively increasing the lactobacillus, promoting direct antimicrobial effect. Besides that, in the literature there is evidence that the organic acids included in the isolated form do not produce an effect on the performance, only the blend of several acids. It is possible that the action of several acids together present synergy among themselves. In the conditions evaluated, the use of a blend of organic acids (1,0 and 2,0 kg t⁻¹) benefited the performance of the broilers at 35 and 42 days.

Key Words: Additives, dietary acidifiers, intestinal health, poultry nutrition

524L Effects of a yeast metabolic bioactive compound and organic selenium on growth performance of broiler chickens. Ricardo V. Nunes*¹, Fabricio Philippsen², Clauber Polese¹, Guilherme Tesser¹, Nilton R. Junior¹, Edinan Hagdon Cirilo¹, Thiago P. Ribeiro³, Natalia Milani³, Daniel Pigatto Monteiro³; ¹*Animal Science, Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon, Paraná, Brazil*, ²*Animal Science, UTFPR, Dois Vizinhos, Paraná, Brazil*, ³*Animal Nutrition, Tectron, Toledo, Brazil*.

The objective of this study was to evaluate the supplementation of a yeast metabolic bioactive (MB) and organic selenium on broilers' growth performance from 1 to 42 d old. Eight hundred male Cobb 500 broilers were used

in a randomized complete block design experiment with 5 treatments, 8 replications per treatment, and 20 birds per experimental unit (cages). The treatments were: T1 - negative control (NC – without MB); T2 - NC + 500 g t⁻¹ of MB; T3 - NC + 1500 g t⁻¹ of MB; T4 - NC + 500 g t⁻¹ of MB + 100 g t⁻¹ of organic selenium; T5 - NC + 1500 g t⁻¹ of MB + 300 g t⁻¹ of organic selenium. The MB used is derived from brewer's yeast, baker's yeast, and sugar cane yeast processing. Growth performance parameters weight gain (WG), feed intake (FI), and feed conversion ratio (FCR) were evaluated at 10, 21, 35, and 42 d. ANOVA was performed and means were separated using the Tukey test (5%). Birds fed T1 and T2 showed 3.6% higher (P<0.05) WG compared to T4 and T5 groups, while T3 was not different (P>0.05) from the other diets on d 10. FI was 4.8% higher (P<0.05) in T3 in contrast the presented by T2, T4, and T5, at the same time that T1 was similar (P>0.05) from them. At d 10 better and 2.3% lower (P<0.05) FCR was observed when T2 was consumed. Birds' performance was not (P>0.05) influenced by the evaluated treatments on d 21 and d 35. FI was 2.6% higher (P<0.05) for birds that received T3 compared to T4, and both did not differ (P>0.05) to T1, T2, and T5 on d 42, while no effects (P>0.05) were observed in WG and FI. On day 10, the improvement noted in the groups that received NC + 500 g t⁻¹ of MB may be related to the early colonization of beneficial yeast having a vital role in the establishment of a favorable microbial environment in the gut which had resulted in better utilization of feed and better absorption of nutrients. Also, might be due to yeast having stimulating action on the digestive tract. In the conditions evaluated, the use of MB benefited the FCR and WG of broiler chickens, while larger conclusions about organic selenium supplementation were not possible.

Key Words: additives, organic minerals, poultry nutrition, prebiotics

525L No presentation materials submitted.

526L No presentation materials submitted.

527L Mineral profile of chicken meat affected by Wooden Breast myopathy stored for 12 months. Erick A. Villegas-Cayllahua*^{GS 1}, Juliana Lolli Malagoli de Mello¹, Rodrigo Fortunato de Oliveira¹, Daniel Rodrigues Dutra¹, Érika Nayara Freire Cavalcanti¹, Mateus Roberto Pereira¹, Rodrigo Alves de Souza², Aline Giampietro-Ganeco², Fábio Borba Ferrari¹, Heloísa de Almeida Fidelis¹, Pedro Alves de Souza¹, Hirasilva Borba¹; ¹*Faculdade de Ciências Agrárias e Veterinárias da UNESP, Jaboticabal, SP, Brazil*, ²*Universidade de Sao Paulo, Pirassununga, SP, Brazil*.

Wooden breast myopathy is known for giving hardness to the pectoral muscle in broilers, in addition to the appearance of petechiae and white stripes, it also alters its chemical composition. The objective of this work was to evaluate the effect of storage on the mineral profile of chicken affected

by Wooden Breast myopathy throughout twelve months of freezing. 360 samples were selected from male chicken breast of Cobb 500 line, slaughtered at 42 days of age, according to the degree of myopathy [severe, moderate and normal (absence of myopathy)]. Samples were classified according to the presence of stiff and pale muscles. Part of the samples (n=60; n=20 for total samples and for each degree of severity) were refrigerated and analyzed on the same day of collection. The remaining samples were packaged and frozen in a freezing tunnel (-40°C), and subsequently stored at -18°C for up to twelve months. The mineral profile analysis was performed by means of nitric-perchloric digestion and the technique of atomic absorption spectrophotometry of mineral matter. For the analysis of the phosphorus present in the samples, the spectrophotometric method of molybdate-vanadate was applied. A completely randomized experimental design in a 3x5 factorial arrangement consisting of 3 degrees of myopathy and 5 storage times (0, 3, 6, 9 and 12 months) was used. The data were analyzed by "One-Way ANOVA" procedure from Statistical Analysis System Software (SAS). The results were submitted to analysis of variance and means compared by Tukey test with significance set at p<0.05. It was observed that samples affected by severe myopathy had lower (p<0.05) concentrations of P (10.37), Mg (0.98) and S (8.65), when compared to normal samples (10.72; 1.06 and 9.08 for P, Mg and S, respectively). Na was the only mineral that increased (p<0.05) its concentration whereas the degree of myopathy increased (2.63 for severe degree) when compared to normal samples (1.94). The storage time caused a decrease (p<0.05) in S values for those samples affected by the myopathy (from 9.16 to 7.09), whereas the difference of its concentration in normal samples was not affected (p>0.05). Lower (p<0.05) mineral values were also observed as the storage time increased for concentrations of Ca (0.288), P (10.7), K (12.51) and Mg (1) at 12 months when compared to time 0 (0.488; 10.92; 13.38; 1.06; 9.12 for Ca, P, K and Mg, respectively), mainly due to the action of ice crystals on the cell membrane, which decrease the water retention capacity of the chicken meat and favor losses of soluble material (such as minerals). It can be concluded that storage causes a decrease in mineral concentrations on chicken meat affected by Wooden Breast myopathy.

Key Words: Calcium, Pectoralis Major, Broiler, Myopathy, Storage

528L Staphylococci profile of Free-Range eggs collected in nest boxes and on the aviary bedding according to the collection time post-laying. Daniel R. Dutra*¹, Nivea Maria G. Misson Carneiro³, Erick A. Villegas-Cayllahua¹, Amanda Cristina M. Silva², Romário A. Rodrigues³, Nadir S. Bornatte⁴, Marco Antonio d. Belo³, Hirasilva Borba²; ¹Animal Science, UNESP, Sertãozinho, São Paulo, Brazil, ²Technology, FCAV/UNESP, Jaboticabal, SP, Brazil, ³Preventive Veterinary Medicine, UNESP, Jaboticabal, SP, Brazil, ⁴FAI Farms, Jaboticabal, Brazil.

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Contaminated eggs with *Staphylococcus* spp., especially coagulase positive enterotoxigenic strains, has increased the cases of foodborne diseases worldwide. However, many studies have suggested that the assessment of coagulase negative staphylococci in fresh eggs should be equally considered, as some of these microorganisms are also toxin producers. Furthermore, the establishment of alternative rearing systems, where birds are cage free and the eggs have contact with the nest and aviary bedding, might increase the risk of contamination. Thus, this study aimed to verify the prevalence of positive and negative coagulase *Staphylococcus* in a batch of approximately 4500 Hisex Brown laying hens, 82 weeks old, reared in a free-range system. 50 eggs were collected, distributed in five times (0, 3, 6, 9, 12 hours post-laying) and two laying locations (nest and aviary bedding). Each pool of samples contained 5 eggs. Shell and internal contents were analyzed. Nest and bedding were also sampled. After quantitative evaluation using the Baird-Parker agar counting method, typical colonies were selected for coagulase test. Despite the current legislation in Brazil (IN60/2019) not to establish permitted staphylococcal parameters for fresh eggs, our results showed absence of coagulase positive *Staphylococcus* in 100% (10/10) of the analyzed eggshell samples. It was also observed that eggshells from the bedding had a higher count of coagulase negative *Staphylococcus* in relation to the ones from the eggs collected in the nest. With advancing the collection time, the count of this group of pathogens on the eggshell from the bedding increased 10⁴ to 10⁶ CFU/g, whereas count in nest eggshells remained stable (10³ CFU/g) throughout 12 hours post-laying. These findings are reflection of the high load of coagulase negative staphylococci present in the nest and on the bed (10⁷ and 10⁸, respectively), which contaminate the outer surface of the eggs. It is known that bacterial load on the shell can affect the penetration of bacteria inside the egg, promoting its deterioration and compromising the consumer's health. However, no staphylococcal contamination of the internal components was found for any of the eggs exposed to the litter and nest. Thus, we conclude that free-range eggs were negative for coagulase positive *Staphylococcus* and had low load of coagulase negative *Staphylococcus* on their shells. However, the need to collect eggs as soon as possible after laying is evident in order to prevent an increase in microbial load on the surface of the eggs, especially those collected on the litter, which should receive differentiated treatment due to their high load of coagulase-negative *Staphylococcus* on its shell.

Key Words: egg microbiology, food safety, free range eggs, public health, Staphylococcus

529L Visual acceptance of chicken meat with different degrees of myopathies. Carlos E. Benito*¹, Laura A. Pinto^{1, 2}, Laura Gubert¹, Luiza R. Stefanello¹, Eduarda P. Simões¹, André N. Pinto¹, Ana C. Ferreira¹, Jovanir I. Fernandes^{1, 2}; ¹Poultry Experimentation Laboratory, Federal University of Paraná, Palotina, PR,

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The study was carried out to evaluate the effect of different degrees of *White striping* and *Wooden breast* myopathies on the consumer's visual acceptance of fresh chicken breast. The severity of the myopathy in the chicken breast meat was classified into 3 groups, with 10 repetitions, namely: normal (NOR), moderate (MOD) and severe (SEV). The breasts were randomly distributed, individually packed and refrigerated display ($4^{\circ}\text{C} \pm 1$; 1200 lux, 12 h day⁻¹). The breasts were randomly removed one by one and photographed each day, for 5 days, for visual assessment and consumer purchase intent. The photographs were produced under standardized conditions following the methodology proposed by Passetti et al. (2017). The photographs were projected randomly and individually on a computer. The visual analysis of the breasts was performed in an appropriate environment with semi-trained evaluators ($n = 25$), who regularly consume chicken meat. The evaluators performed the analyzes of 120 photos in total, being: 8 chicken breasts; 3 types of meat anomalies and 5 days of commercial exposure, which corresponded to the samples in randomized order. The evaluation of the meat's color acceptability was made using the 9-point hedonic scale without the central point. The data were submitted to unilateral ANOVA and performed with the aid of the IBM Statistical Package for the Social Science. Then, visual attributes were evaluated considering the degree of each myopathy and exposure time as fixed effects. Similarly, in the evaluation of consumer's acceptability, the degree of each myopathy and exposure time were considered as fixed effects and the consumer was included as a random effect. To analyze the number of days, regressions were performed between exposure days and acceptability scores. The differences were evaluated by Tukey's test ($P < 0.05$). According to our findings, visual acceptance was different between meat types. During the period of visual assessment of breasts with the presence of myopathies, consumers gave higher scores to NOR meats ($P < 0.001$). The maximum exposure time in which consumers rated meats with positive scores (above 5) was 2.4 days for NOR breasts, 1.22 for MOD breasts and 1.77 days for SEV breasts; after this period were not accepted by consumers. In relation to exposure time, the intention to buy the breasts decreased over the days. The results of this study showed that the presence of myopathies affects the consumer's visual acceptance of refrigerated chicken meat. Considering that the consumer's visual acceptability has direct effects on the purchase intention, a better understanding of the effect of myopathies is essential to assess consumer preferences at the time of purchase.

Key Words: Consumer, Chicken meat quality, visual acceptability, Wooden breast, White Stripe

530L The effect of clove essential oil as a natural preservative in the oxidation of chicken hamburgers. Laura Gubert^{*1}, Carlos E. Benito¹, André Pinto¹, Luiza R.

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We evaluated the effects of including the essential oil (OE) of clove as a possible substitute for the synthetic butylated hydroxytoluene (BHT) additive in frozen chicken hamburgers. Analyzes of antioxidant capacity (ABTS and TPC) and lipid oxidation were performed. The chicken breast meat was obtained in a single batch, from a commercial poultry processing unit in the western region of Paraná state, Brazil. After slaughter, Pectoralis major (PM) was removed from the carcasses and cooled ($4^{\circ}\text{C} \pm 1$ for 5h). After that, the breasts were packed in plastic bags and transported to the poultry experimentation laboratory at the Federal University of Paraná – Palotina, where they were frozen ($-5^{\circ}\text{C} \pm 1$) until the time of analysis (3 months of storage). The treatments were defined as follows: addition of 0.01% clove essential oil (CEO), 0.01% BHT (CON), and without the addition of antioxidant (STD). The BHT concentration used in this study followed the standards established by the Ministry of Health from Brazil for meat products (100 mg/kg of product). The breasts were thawed under refrigeration ($4^{\circ}\text{C} \pm 1$), grounded (industrial meat grinder with 6 mm mesh). To manufacture the hamburgers, all ingredients were homogenized manually (10 min) and the hamburgers were molded as patties of 80 g, 10 cm in diameter and 2.0 in height. The samples were individually packed in retractable polystyrene bags permeable to oxygen and taken in a freezer ($-10^{\circ}\text{C} \pm 1$). For the analysis, the hamburgers from each treatment (8 replicates in triplicate) were randomly removed on days 1 and 90 from the frozen storage. The phenolic content (TPC), the antioxidant activity and the lipid oxidation of the hamburgers were analyzed and the results were expressed as mg of gallic acid equivalent (GAE)/g hamburger. The percentage of radical reduction (ABTS*) activity was expressed in % of reduction. To assess lipid oxidation, a sample of hamburger (5 g) was mixed with a TCA solution and the results were expressed in mg MDA/kg of hamburger. The experimental design was completely randomized, in triplicate and three storage times (5 days). The significance of the differences between treatments was made by ANOVA and Tukey as a post hoc test. Our results showed that the addition of clove EO had an antioxidant effect on frozen chicken hamburgers ($P > 0.05$), reducing lipid oxidation processes during freezing. The essential oil showed the highest antioxidant activity and phenolic content at the end of storage. Therefore, clove essential oil has been a promising natural antioxidant to increase the shelf life of meat products, as well as a viable solution to replace the synthetic antioxidant BHT and other commercial additives widely used by the food industry.

Key Words: Antioxidant, butylated hydroxytoluene, Clove essential oil, lipid oxidation, meat quality

531L The effect of different degrees of wooden breast and white stripe myopathies on the quality of refrigerated chicken meat. Luiza R. Stefanello¹, Regina Buzim^{*2}, Juliana S. Schuroff¹, Julianne R. Severiano¹, James Barbosa¹, Carlos E. Benito¹, Laura A. Pinto^{3, 1}, Jovanir I. Fernandes^{1, 2, 3}; ¹*Poultry Experimentation Laboratory, Federal University of Paraná – Sector Palotina, Palotina, PR, Brazil,* ²*Animal Science Post-Graduate Program, State University of Paraná, Marechal Cândido Rondon, Marechal Cândido Rondon, Paraná, Brazil,* ³*Animal Science Post-Graduate Program, Federal University of Paraná- Sector Palotina, Palotina, PR, Brazil.*

This study aims to evaluate the effect of the presence of *Wooden breast* and *White stripe* myopathies on the quality attributes of refrigerated chicken meat. Chicken breast meat was obtained in a single batch from a commercial unit in the western region of Parana state, Brazil. The severity degree of myopathies in chicken breasts was performed by visual examination and palpation of the *Pectoralis major* (PM) muscle, into three groups: normal (NOR), moderate (MOD) and severe (SEV). The PMs were packed one by one in a polystyrene tray wrapped with a retractable film, stored refrigerated to simulate typical Brazilian market ($4\pm 1^\circ\text{C}$, 1200 lux, 12h day⁻¹). At 1, 3 and 5 days of storage, 30 samples (10 per treatment) were randomly removed for the meat quality analysis. The breasts were evaluated regarding their lipid oxidation (mg MDA/kg of meat), pH, cooking loss (water bath at 80°C and cooked until the internal temperature reached 78°C), shear force (measured using a texture analyzer), and the most microbiological analysis to coliform (coliforms at 45°C, (NMP)/g of sample). Statistical analyzes were performed using SPSS version. The experimental design was completely randomized, with three treatments (NOR, MOD and SEV) in triplicates and three storage times (1, 3 and 5 days). The significance of the differences between

treatments and storage times was confirmed using the Tukey test at $P<0.05$. The oxidation of the samples was influenced by the degree of myopathies ($P<0.004$) and storage ($P<0.001$). Breasts with myopathies showed higher lipid oxidation compared to normal breasts. In addition, lipid oxidation enhanced with increasing storage time ($P<0.001$). Breasts with presence of myopathies (MOD and SEV) had higher pH values ($P<0.004$). However, during storage, the pH did not change ($P>0.05$). The presence of severe myopathy (SEV) provided greater losses during cooking. In addition, cooking losses enhanced ($P<0.001$) with increasing storage time. The presence of myopathies had an effect ($P<0.001$) on the shear force of breasts. NOR breasts had lower shear values compared to MOD and SEV, which did not differ from each other. However, storage did not change the tenderness of the breasts ($P>0.05$). All breasts (NOR, MOD and SEV) had counts below the limit (limit of $5 \log^{10}$ CFU/g of coliforms at 45°C) on day 01 of storage, and starting on day 03, the MOD breasts have already exceeded the limit microbiological acceptable for refrigerated chicken meat. Our findings show that the presence of myopathies has a negative impact not only on the visual appearance of the meat, but they can directly impact on the technological and microbiological properties of refrigerated meat, preventing its commercialization in this form.

Key Words: lipid oxidation, myopathies, meat quality, shelf life, technological properties

532L No presentation materials submitted.

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