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Abstracts

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## SYMPOSIA AND ORAL SESSIONS

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Animal Well-Being and Behavior

1 The gut microbiota-brain axis: Modifications of injurious behaviors and physiological homeostasis in chickens through early postnatal cecal microbiota transplantation. Yuechi Fu<sup>*</sup>, Jiaying Hu<sup>1</sup>, Heng-wei Cheng<sup>3</sup>, 1Purdue University, West Lafayette, Indiana, United States, 2Livestock Behavior Research Unit, USDA-ARS, West Lafayette, Indiana, United States.

Fecal microbiota transplantation (FMT) has been used as a bacteriotherapy for major mental illness in humans. The aim of this study was to determine if cecal microbiota transplantation (CMT) modifies growth performance and injurious behaviors (i.e., severe feather pecking and aggressive pecking) in chickens through the regulation of the gut microbiota-brain axis. Cecal bacterial samples were collected from hens of 63 and 72 strains which were divergently selected for resistance to Marek’s disease, resulting in the lines’ unique biological characteristics and behavioral expression, as 63 birds are more gentle than 72 birds. The cecal bacterial samples were diluted at 1:10 with gut microbiome media before oral gavage. A total of 189 d-old chicks of an aggressive Dekalb XL line were randomly assigned to 1 of 3 treatments in 7 replicates (n=7): oral gavage of 0.1 mL saline (CONT), 0.1 mL cecal bacterial solution of 63 strain (63-CMT), and 0.1 mL cecal bacterial solution of 72 strain (72-CMT). Birds were fed once a day from wk 1 to 10, and then once per wk at wk 3, 4 and 5. Roosters and pullets were separated at 5 wk of age and then kept in cages with 4 birds per cage. Body weight of roosters was recorded at wk 5, 10, and 16; and pullets were weighed at wk 9, 16, 20, and 30. At wk 16, the spleen, liver and left adrenal gland were collected from 1 rooster per cage, and relative organ weight was calculated. At wk 20, hens with similar BW were assigned to paired aggression tests between treatments. Two-way ANOVA, one-way ANOVA, and non-parametric analysis were used for BW, organ weight, and paired aggression tests analysis, respectively. Significance was set at P < 0.05 and trends were defined as 0.1 ≥ P ≥ 0.05. The results showed that 72-CMT roosters had a greater BW than both control and 63-CMT roosters (P<0.0001); while 72-CMT pullets had a greater BW than controls (P < 0.0001) but not 63-CMT pullets (P > 0.05). Pullets of 63-CMT had a tendency of less severe feather pecking during the paired aggression test compared to both 72-CMT and control pullets (P = 0.1). In addition, 63-CMT pullets had lighter adrenal glands than both 72-CMT and control pullets (P < 0.05). There was no treatment effect on spleen and liver weight (P > 0.05). The results indicate that early postnatal CMT may affect recipient chickens’ growth performance and injurious behavioral expression.

Key Words: gut microbiota-brain axis, injurious behavior, cecal microbiota transplantation, growth, chickens

2 Omega-3 in maternal diet improves cognitive performance of broiler chicks in a T-maze test. Rosemary Whittle<sup>*</sup>, Elijah Kiarie, Tina Widowski, University of Guelph, Guelph, Ontario, Canada.

Adding flaxseed to breeder feed primes the developing egg with omega-3s (n-3s) necessary for healthy embryonic development. Neural functions dependent on n-3s include neurogenesis and myelination, therefore supplementing n-3s could increase cognitive function. We hypothesised that maternal fed n-3s will increase the number of successful trials in a T-maze test for broiler chicks. Broiler breeders were fed control (C) or “LinPro” flaxseed enriched (L) maternal rearing diet (MRD) or maternal laying diet (MLD), creating four rearing-laying maternal diet combinations (MDC), control-control (C-C), control-LinPro (C-L), LinPro-control (L-C) and LinPro-LinPro (L-L). The breeders were housed in 8 pens, with 2 pens per MDC. Male (M) and female (F) broiler chicks were hatched across two replicates, in the same commercial incubator and hatcher at the University of Guelph poultry research station. The eggs of the second replicate were set 3.5 weeks after those from the first replicate. Broiler chicks were housed in mixed-sex groups of 20 chicks (from the same parental unit), in 2 rooms per replicate, each containing 12 floor pens following a randomised complete block design. A total of 288 chicks, (72 per MDC, 6 per pen), balanced for sex, were tested in a T-maze test at 8-10 days old for five trials. The results were analysed using general linear mixed effects models, using pen as the experimental unit. Broilers from L MLD had significantly more successful trials in the T-maze than C MLD (t=-2.431, p=0.0157). There was no effect of MRD on the number of successful trials (t=-0.723, p=0.4701). Pairwise comparisons of MRD and MLD showed a tendency for C-L MDC to have more successful trials than C-C (t=2.431, p=0.0737) and L-L MDC to be more successful than L-C (t=2.431, p=0.0737). Pairwise comparisons of MLD and sex showed L-F had significantly more successful trials in the T-maze test than C-M (t=2.765, p=0.0307), L-F tended to have more successful trials than C-F (t=2.431, p=0.0737) and L-M tended to have more successful trials than C-M (t=2.431, p=0.0737). The results show maternal fed n-3s could increase cognitive function of broiler chicks in T-maze tests, especially in the MLD. Increased T-maze performance could be attributed to increased availability of n-3s for neural development. This supports the hypothesis that maternal fed n-3s can increase cognitive ability of broiler chicks.

Key Words: behaviour, cognition, nutrition, omega-3, maternal effects

3 Tryptophan metabolism, Stress and Feather Pecking in Pullets. Claire Mindus<sup>*</sup>, Nienke van Staaveren<sup>1</sup>, Paul Forsythe<sup>2</sup>, Simon Geisler<sup>3</sup>, Johanna M. Gostner<sup>1</sup>, Joergen B. Kjaer<sup>4</sup>, Wolfgang Kunze<sup>2</sup>, Anna Kate

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<sup>★</sup> Undergraduate Student Presenter
In mammals, the metabolism of the indispensable amino acid tryptophan (TRP) can be influenced by stress, leading to an increase of TRP breakdown towards the kynurenine (KYN) pathway. In laying hens, feather pecking (FP) is a destructive behavioural disorder occurring when one individual forcefully and repetitively pecks at the plumage of conspecifics. FP can be triggered by stress and is associated with disruptions in TRP catabolism in adult laying hens. In pullets, however, the TRP catabolism along the KYN axis may differ and respond differently to stress.

As a part of a larger project, we investigated the effect of Unpredictable, Repeated Stressors (URS) on plasma concentrations of TRP and KYN in female pullets housed in enriched floor pens. White Leghorn pullets (n=354, 10 weeks of age) originated from 3 different lines of birds bred for their high, low or unselected FP phenotype (120±2 birds/line). The birds were incubated, hatched, individually tagged and reared together on the research farm site. Immediately after hatch, these birds were systematically assigned to 6 stressed (S) or 6 non-stressed pens (NS) (10±1 birds/line/pen) systematically distributed across one room. The 6 S groups were exposed to social (i.e. mixing with unfamiliar birds, holding in a transport crate in a crouched position for 1 hour) and non-social (i.e. introduction to a novel environment, removal of nest boxes or perches) URS from 11 to 13 weeks of age, while the NS groups were left undisturbed. Blood samples were collected one-hour post-feeding before and after the 3 weeks of URS, and analyzed using high performance liquid chromatography. A generalized linear mixed model was used to assess the effect of the genetic lines and URS on plasma concentrations of TRP, KYN as well as their ratio, calculated as an indication of the genetic lines and URS on plasma concentrations of TRP, KYN and their ratio, calculated as an indication of the activity of TRP catabolism. The birds were considered as the experimental unit. Our results revealed that birds from the line bred for its low FP phenotype showed significantly higher levels of plasma TRP (P<0.001) compared to birds bred for their high FP phenotype. TRP (+6%, P<0.001), KYN (+33%, P<0.001) and their ratio (+26%, P<0.001) significantly increased over the URS period for all birds, regardless of their URS treatment. The URS treatment did not significantly impact the TRP, KYN or ratio (P>0.05). These results suggest that differences in aromatic amino acid metabolism already exist in pullets bred for extreme FP phenotypes. Together, these underlying differences may help to predict different dietary and environmental needs of FP and non-FP pullets and contribute to our understanding of early phenotypic markers for FP.

Key Words: pullet, stress, feather pecking, tryptophan, metabolism

4 Effect of light intensity on calcium homeostasis in pullets. Gula Sadvakassova1, 2, Jo Ann Chew3, Kailyn Beaulac3, Hossein Poorhemati2, 4, Karen Schwean-Lardner1, Svetlana V. Komarova1, 2, 4, McGill University, Montreal, Quebec, Canada, 1Shriners Hospital for Children, Montreal, Quebec, Canada, 2University of Saskatchewan, Edmonton, Alberta, Canada, 3McGill University, Montreal, Quebec, Canada.

The impact of varying light intensities on layer pullets is not yet well understood. Behaviorally, brighter illumination may improve pullet activity levels by allowing better navigation in the complexity of non-cage systems. In addition, light intensity was previously demonstrated to affect the levels of calcium and phosphate regulating hormones in mice. The objective of this study was to examine how exposure of pullets to the light of different intensity affects their calcium and phosphorus homeostasis. Lohmann LSL-Lite and Lohmann Brown-Lite pullets were randomized into four individually controlled rooms, each containing 6 pens per room which were assigned to 10 or 50 lux light intensity supplied via white LED lighting (2821K) during the photophase. Plasma samples were collected after 8 and 16 weeks of exposure. Calcium, phosphorus and magnesium were measured by inductively coupled plasma optical emission spectrometry; parathyroid hormone (PTH), calcitriol (1,25-dihydroxyvitamin D, 1,25(OH)2D) and fibroblast growth factor 23 (FGF23) were measured by ELISA. The data were analyzed by one-way ANOVA. Levels of calcium, magnesium and phosphorus were similar in Lohmann LSL-Lite and Lohmann Brown-Lite pullets. Total plasma calcium and magnesium were significantly higher (p<0.05) in 16-week old compared to 8-week old pullets of both breed, but were not affected by the light intensity. Plasma phosphorus was unaffected by breed, age or light intensity. PTH, calcitriion and FGF23 were similar in 8-week old Lohmann LSL-Lite and Lohmann Brown-Lite pullets. PTH significantly (p<0.01) decreased with age in both breeds, but to significantly (p<0.01) lower levels in Lohmann Brown-Lite compared to Lohmann LSL-Lite pullets. Calcitriol and FGF23 significantly (p<0.01) increased with age in Lohmann LSL-Lite, but not in Lohmann Brown-Lite pullets. Young, 8-week old Lohmann Brown-Lite pullets demonstrated a trend (0.05<p<0.1) of increased PTH and Calcitriol at 50 lux compared to 10 lux. Thus, calcium regulating hormones, as well as plasma calcium and magnesium levels significantly change with age in both Lohmann LSL-Lite and Lohmann Brown-Lite pullets. Light intensity may additionally affect hormones regulating calcium homeostasis, however further experiments are needed to verify the changes.

Key Words: pullets, egg-laying, light intensity, calcium, phosphorus
5  The effects of rearing cage type and calcium particle size on keel bone characteristics of laying hens. Madeleine Browne1,2, Tanka Khanal1, Tina Widowski1, Elijah Kiarie2, ‘Chicken Farmers of Ontario, Burlington, Ontario, Canada, 1Animal Biosciences, University of Guelph, Guelph, Ontario, Canada.

Keel bone damage (KBD) is a major welfare concern in commercial egg production. Key aspects of hen development, such as environment and nutrition, may effectively reduce incidences of KBD later in production. Larger, more complex environments allow for exercise that improves muscle deposition and bone mineralization. Similarly, dietary calcium is critical for hen bone formation and integrity, especially prior to the onset of lay. This study investigates the effects of rearing cage type and calcium (limestone) particle size (PS) fed during rearing on laying hen keel bone characteristics at 16, 24 and 40 weeks of age (woa). It was hypothesized that rearing pullets in enriched cages and provided a larger PS will improve adult keel bone characteristics at 16, 24 and 40 weeks of age (woa). A total of 900 cages and provided a larger PS will improve adult keel bone characteristics at 16, 24 and 40 weeks of age (woa). It was hypothesized that rearing pullets in enriched cages and those reared in EC (80%) compared to CC (43.7%). Pullets reared in EC also had higher keel ash contents (43.7%) raised under BL. The results indicate that exposure to blue light results in minor differences in refraction and spatial vision. However, it is unclear whether behavioral changes are associated with alterations to the bird’s visual system and spatial vision. To assess the impact of raising broilers under 3 colour light (L) treatments (blue (BL, 455nm), green (GL, 510 nm) or white (WL)) on eye health and spatial vision, Ross 708 males were assessed in 3 experiments. In experiment 1, a complete ophthalmic examination was performed to assess eye health in 36 birds at 21 days of age (12 per L), which included chromatic pupillary light reflex (PLR) testing, rebound tonometry, anterior segment biomicroscopy, and indirect ophthalmoscopy. In order to assess ocular anatomy, in experiments 1 and 2, 60 birds (17 days of age) were euthanized, both eyes were enucleated, weighed (N=108 birds, 36 per L), and dimensions were collected (corneal diameter, mediolateral diameter, dorsoventral diameter and anteroposterior size) using a digital caliper. In experiment 2, an autorefractor was used to assess the corneal curvature and the refractive index in 18 birds at 26 days of age (6 per L). To assess spatial vision, in experiment 3, 24 birds at 29 days of age raised under BL or WL (12 per L) underwent a grating acuity test under three different distances (50, 75 and 100 cm). Data were statistically analysed using SAS Proc Mixed or Proc Par1way for data not normally distributed (SAS 9.4) as a one-way factorial analysis. Birds were the experimental units. No significant differences were observed for tonometry (P=0.74) and no abnormalities were revealed during chromatic PLR testing, biomicroscopy, and indirect ophthalmoscopy. Eye weights and dimensions did not differ by light treatments. For autorefraction, birds raised under BL had a higher sphere index (0.625) than birds raised under WL (-0.020, P=0.01) however, no differences were observed for cylinder and axis indices. Light treatment may have affected the spatial vision of broilers, as birds raised under BL approached the stimulus faster at the distances of 50 cm (P=0.03) and 75 cm (P=0.006) and had a higher success rate (P=0.03) at the distance of 100 cm than birds raised under WL. The results indicate that exposure to blue light results in minor differences in refraction and spatial vision. However, it is not clear if these differences alone are sufficient to fully explain the substantial changes in bird behavior that have been observed.

6  The effect of light wavelengths on broilers: more than meets the eye. Bruna Maria Remonato Franco1,2, Marina Leis1, Melody Wong1, Troy Shynkaruk1, Bryan Fancher1, Nick French1, Scott Gillingham1, Karen Schwean-Lardner1, ‘Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 1Department of Small Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 1Department of Ophthalmology, Saskatoon City Hospital, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 1Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 1Aviagen, Huntsville, Alabama, United States.

Varying wavelength is an option for broiler production, and differing wavelengths (color) can lead to alterations in bird’s behavior. However, it is unclear whether behavioral changes are associated with alterations to the bird’s visual system and spatial vision. To assess the impact of raising broilers under 3 colour light (L) treatments (blue (BL, 455nm), green (GL, 510 nm) or white (WL)) on eye health and spatial vision, Ross 708 males were assessed in 3 experiments. In experiment 1, a complete ophthalmic examination was performed to assess eye health in 36 birds at 21 days of age (12 per L), which included chromatic pupillary light reflex (PLR) testing, rebound tonometry, anterior segment biomicroscopy, and indirect ophthalmoscopy. In order to assess ocular anatomy, in experiments 1 and 2, 60 birds (17 days of age) were euthanized, both eyes were enucleated, weighed (N=108 birds, 36 per L), and dimensions were collected (corneal diameter, mediolateral diameter, dorsoventral diameter and anteroposterior size) using a digital caliper. In experiment 2, an autorefractor was used to assess the corneal curvature and the refractive index in 18 birds at 26 days of age (6 per L). To assess spatial vision, in experiment 3, 24 birds at 29 days of age raised under BL or WL (12 per L) underwent a grating acuity test under three different distances (50, 75 and 100 cm). Data were statistically analysed using SAS Proc Mixed or Proc Par1way for data not normally distributed (SAS 9.4) as a one-way factorial analysis. Birds were the experimental units. No significant differences were observed for tonometry (P=0.74) and no abnormalities were revealed during chromatic PLR testing, biomicroscopy, and indirect ophthalmoscopy. Eye weights and dimensions did not differ by light treatments. For autorefraction, birds raised under BL had a higher sphere index (0.625) than birds raised under WL (-0.020, P=0.01) however, no differences were observed for cylinder and axis indices. Light treatment may have affected the spatial vision of broilers, as birds raised under BL approached the stimulus faster at the distances of 50 cm (P=0.03) and 75 cm (P=0.006) and had a higher success rate (P=0.03) at the distance of 100 cm than birds raised under WL. The results indicate that exposure to blue light results in minor differences in refraction and spatial vision. However, it is not clear if these differences alone are sufficient to fully explain the substantial changes in bird behavior that have been observed.
7 Differences in feather corticosterone level between purebred turkey lines with different breeding objectives. Emily M. Leishman\textsuperscript{a}\textsuperscript{c}, Nikole E. Freeman\textsuperscript{b}, Amy E. Newman\textsuperscript{b}, Nienke van Staaveren\textsuperscript{a}, Ben J. Wood\textsuperscript{a}\textsuperscript{b}\textsuperscript{c}, Alexandra Harlander\textsuperscript{d}, Christine F. Baes\textsuperscript{a}\textsuperscript{e}, ‘Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, ‘Integrative Biology, University of Guelph, Guelph, Ontario, Canada, ‘Hybrid Turkeys, Kitchener, Ontario, Canada, ‘School of Veterinary Science, University of Queensland, Gatton, Queensland, Australia, ‘Institute of Genetics, University of Bern, Bern, Switzerland.

Measuring corticosterone in feathers is becoming a frequent non-invasive method for assessing glucocorticoid levels in poultry. The objective of this study was to assess the effectiveness of detecting corticosterone in turkey feathers using an ELISA and to investigate the potential for differences in genetic lines. Primary wing feather nine was collected at a processing plant from 123 individual toms from three different purebred turkey lines (line A (N=46), B (N=24), and C (N=53)). Lines A and B are selected for reproductive traits whereas Line C is selected for growth and production traits. All individuals within and between lines were housed under identical husbandry conditions. Individual samples were analyzed using a commercially available ELISA kit (Cayman Chemicals, Cedarlane Labs, Canada). Additional validation tests for accuracy, precision, and steroid recovery were conducted using a species pool of pulverized feathers from different individuals (N=7). Accuracy was determined using an ANCOVA to assess if there was a significant interaction between the standard curve and serial dilution of pooled turkey feathers. Steroid recovery was determined by spiking the species pool with a known quantity of corticosterone. Precision was measured through intra- and inter-assay variability. To test for differences in feather corticosterone concentration between genetic lines, a one-factor ANOVA was used. A post-hoc Tukey HSD test was then performed to further investigate the pairwise comparisons between lines. This study showed that feather corticosterone could be measured in the primary wing feathers of domesticated turkeys via an ELISA. There was no significant interaction between the standard curve and serial dilution which indicates adequate specificity of the assay (ANOVA: \(P=0.267\)). Average recovery of corticosterone was 65% and the intra- and inter-assay CV of the ELISAs were 1.4 ± 2.48% and 12.1 ± 0.02%, respectively. Significant differences were observed in the feather corticosterone concentrations between the three lines (ANOVA: \(P<0.001\)). Line C had significantly higher feather corticosterone than Line A (Tukey HSD: \(P=0.0001\)) and Line B (Tukey HSD: \(P=0.036\)). The feather corticosterone levels were not significantly different between Line A and Line B (Tukey HSD: \(P=0.366\)). This study is the first to examine differences in feather corticosterone between different purebred turkey lines. Since all individuals were raised in identical conditions, the differences found may be the result of the different breeding objectives of each purebred line and allows for the further investigation into the underlying genetics of glucocorticoid levels in turkeys.

Key Words: vision, LED, light color, behaviour, welfare

8 Maternal age and housing system of laying hens interact to affect measures of fear and stress in their offspring. Mariana R. Peixoto\textsuperscript{a}, Leanne Cooley\textsuperscript{b}, Tina Widowski\textsuperscript{c}, ‘Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, ‘L.H. Gray & Son Limited, Strathroy, Ontario, Canada.

Maternal effects can shape the phenotype of offspring, but the extent to which a layer breeder’s experiences affect laying hens remains unclear. This study further investigates influences of maternal age, rearing and housing environments on measures of fear and stress in their offspring. One cohort of LSL-Lite Commercial white leghorns (N=678) were reared either in aviary (AV) (n=2) or conventional rearing cages (CC) (n=12) from day 1, then housed in aviary (AV) (n=2), conventional layer cages (CC) (n=12) or large 60-bird furnished cages (FC) (n=12) from 16 weeks of age, forming five “rearing X housing” treatments: Trt1 (AV x AV), Trt2 (CC X CC), Trt3 (AV x CC), Trt4 (CC X FC), and Trt5 (AV X FC). Hens from each treatment (n=96) were inseminted with pooled semen at 3 ages: Young (25 weeks), Ideal (44 weeks) and Old (68 weeks). Eggs were incubated, hatched, and offspring (N=240) brooded under identical conditions in furnished pens of 14 birds distributed across 2 rooms. At 9 weeks, offspring were subjected to a manual restraint test, where an individual bird was taken out of its home pen and manually restrained on its side for 5 min, followed by blood sample collection for plasma corticosterone (CORT) concentration. The frequency of struggling during the test was recorded. Fixed effects of sex, maternal treatment (Trt), maternal age and maternal Trt by age interaction were tested using a generalized linear mixed model in SAS. Random effects included pen nested within room and person applying the test. Contrast comparisons tested effects of maternal rearing experience (AV or CC) and maternal housing (AV, CC or CF). Results show an interaction between Trt and maternal age for CORT (\(P=0.0025\)) and frequency of struggling (\(P=0.0025\)). Within Ideal maternal age, Trt1 (1.20 ± 0.1) had the lowest CORT concentration and differed from Trt2 (1.82 ± 0.2; \(P=0.048\)) and Trt5 (2.18 ± 0.2; \(P=0.001\)). Within Young maternal age, Trt1 (1.77 ± 0.5) struggled more than Trt4 (0.59 ± 0.2; \(P=0.048\)). Contrast comparisons showed effects of maternal housing experience, but no effects of maternal rearing. Mothers housed in AV had offspring with lower CORT than mothers in CC (\(F=3.59\); \(P=0.060\)) or FC (F=5.19; P=0.024). Mothers housed in AV also had offspring that struggled more during restraint than those from CC (\(F=3.09\); P=0.080). Results suggest that the age and housing experience of a layer leghorn breeder can affect the physiology and behaviour of the offspring, and

Key Words: feather corticosterone, ELISA, turkey, welfare, genetics
that breeders reared and housed in aviaries may have offspring that produce less CORT and behave more actively in response to a stressor. This data also suggests that maternal housing may have a stronger effect on the analyzed traits than maternal rearing experience.

**Key Words:** layer breeder, laying hen, maternal effects, housing, behaviour

9 Male familiarity and aggressive behavior: two modulators of female Japanese quail social preferences.

Stefania Pellegrini1, Diego A. Guzman2, Raul H. Marin1,2, *Universidad Nacional de Córdoba, Facultad de Ciencias Exactas, Físicas y Naturales, Instituto de Ciencia y Tecnología de los Alimentos (ICTA), Cordoba, Argentina, 2Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Investigaciones Biológicas y Tecnológicas (IIByT, CONICET-UNC), Cordoba, Argentina.

Sexually experienced female quail that have observed an aggressive interaction between a pair of males prefer the less aggressive male, while females with no previous sexual experience prefer the aggressive one. Although those studies were developed in a setup where birds can only interact through a glass separation (no physical contact), social proximity was discussed in terms of reproductive preferences. Another factor that modulates the birds’ choice to interact with conspecífics is the familiarity with other birds. Herein we assessed whether female quail will differentially modulate their social interactions with known or unknown males after observing them taking a high vs. a low aggressive role in a male-male encounter (4 experimental group combinations). Birds were housed in male-female pairs during the rearing period and all females tested were sexually experienced. At 100 d of age, 2 males were tested during up to 2 hs in the presence of their 2 female partners that remained as audience behind a wire partition in two separated compartments (27 total interactions). After the male-male encounters, males were classified as either high or low aggressive. Then (24 hs), the female interactions with those males were individually evaluated during 2 hs in a novel environmental setup that combined the two males fitted with an individual physical barrier (IPB) on their back, the female with no IPB and gated partitions within the apparatus. Thus, only females can freely ambulate through the gates and visit all compartments. This novel social test allows females to choose between remaining separated from each of the 2 males that are restrained in opposite sides of the apparatus, or to enter their environment and physically interact with them. Differences in the time spent with or near each male and numbers of mating and aggressions were analyzed using mixed GLM. Females spent more time (P<0.003) near their known male partner than with the unknown male regardless of the male aggressiveness observed, suggesting that familiarity strongly favors female social reinstatement. However, females copulated equally (P=0.53) with both males regardless of the male familiarity or aggressiveness shown during male-male encounters. Finally, females behaved more aggressively than males and were even more aggressive towards the unknown males. Furthermore, females were more aggressive (P<0.001) towards males that were highly aggressive during the male-male interactions than towards the males that were less aggressive. Taken together, the observed female social behavior suggests that the time spent near a conspecific or the aggressiveness performed against them cannot be used as reliable indicators of sexual preference.

**Key Words:** Japanese quail, social preferences, bird familiarity, aggressive behavior, reproductive behavior

10 Circadian Rhythm of Dust Bathing in 4 Strains of Laying Hen.

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Producers are moving towards tiered aviary systems to house laying hens. These aviaries include multi-level wire enclosures and provide litter areas on the floor. Some aviaries have doors that can confine the hens within the tiered enclosure, in most cases to promote oviposition in nests to prevent eggs being laid in litter. However, there are multiple genetic strains of laying hen used in the egg industry, and some show different circadian patterns for key behaviors. For example, though dust bathing by laying hens typically peaks in early afternoon, there may be variation in timing of dust bathing among strains. Differences in laying hen behavior patterns, coupled with standard aviary set-ups or typical management practices, may make it difficult to suitably allow birds the freedom to perform certain important behaviors, such as dust bathing, while preventing negative behaviors such as laying eggs in litter. This study is a first look at the circadian rhythm of dust bathing in different strains of laying hens. Our objective was to determine if there were strain differences in the behavioral pattern of dust bathing. We examined the circadian rhythm of dust bathing in 4 strains of laying hen (Hy-Line Brown [HB], Bovans Brown [BB], DeKalb White [DW] and Hy-Line W36 [W36]) housed in aviaries and separated by strain. There were 144 hens of each strain in each aviary unit (4 units/strain), and litter access was provided for each unit from 11:30 A.M. to 1:00 A.M. We video recorded hens on the litter at 28 weeks of age from 11:30 A.M., when doors opened, until 8:00 P.M. at lights off. We recorded the number of hens dust bathing and on the litter from this video using instantaneous scan sampling every 2.5 minutes. Descriptive analysis was done by visually evaluating graphs showing number of hens dust bathing superimposed over the number of hens on litter at that time. Hens of white strains were more likely to dust bathe as soon as they gained access to litter compared to the brown strains. In the first
thirty minutes after doors opened, an average of 10.3 DW hens and 9.3 W36 hens were dust bathing, compared to 2.3 BB hens and 0.9 HB hens. One-way ANOVAs showed that hens of white strains were more likely to dust bathe simultaneously and in the presence of more and closer conspecifics compared to brown strains (P<0.01). Brown hens dust bathed in lower numbers (P<0.01) and generally had fewer dust bathing bouts across the day with small peaks in the early afternoon. Further examination of circadian rhythm of behaviors, such as dust bathing, under unconstrained conditions by a range of genetic strains of laying hens is needed to design management practices and aviary styles that best meet hens’ needs.

Key Words: laying hen, dust bath, circadian rhythm, strain, behavior

11 Comparison of electrocardiogram parameters during two methods of euthanasia in white leghorn hens.
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This study compared two common methods of euthanasia (cervical dislocation and CO2 displacement) for differences in heart rate and QRS amplitude in sixteen Bovans White Leghorn laying hens (10-12 months of age). The hens were implanted subcutaneously with wireless telemetry devices two months prior for another study already requiring euthanasia at its conclusion. Cervical dislocation (CD) was carried out manually. For the CO2 procedure the hen was placed in a clear plastic chamber (23"l x 16.25"w x 12.375"h) and gas was added at a 30% displacement rate. Continuous electrocardiogram data were collected before the procedure to establish a baseline for each hen, then continued after euthanasia until cessation of cardiac activity. Insensibility and confirmation of successful euthanasia were determined by the handler to be loss of posture and the end of neuromuscular convulsions with no noticeable breathing (average elapsed time in minutes CD=1:48; CO2=2:39). The procedures were monitored by the attending veterinarian who evaluated the hens via auscultation of the heart immediately after the handler’s confirmation, to provide a second means of confirmation. Electrocardiogram parameters of interest were heart rate and total QRS complex amplitude at baseline, start of procedure, and the following 5 minutes in 1-minute increments. A sample of ten QRS complexes at each time point was used for calculations. It was hypothesized that both heart rate and amplitude would be higher following CD compared to CO2. Data were adjusted for repeated measures, analyzed using the Glimmix Procedure in SAS 9.4, and reported as deviations from baseline. For heart rate there was a significant effect for method (p=0.0148), time (p=0.0001), and method*time interaction (p=0.0001). Compared to baseline, heart rate for CD was 237.62bpm higher at minute 1 (p=0.0001) and 106.35bpm higher at minute 2 (p=0.0019) than CO2. This is likely due to an increased sympathetic response due to the stress of CD. While there was no significant effect of method for QRS amplitude (p=0.6097), there was a time effect (p=0.0344). This shows that the strength of cardiac muscle contraction followed the same pattern regardless of method. Amplitude increased from baseline to the start of the procedure by 0.07875mV, approaching significance (p=0.0658). Compared to the start of the procedure amplitude was 0.1269mV lower at minute 2 (p=0.0044), 0.1469mV lower at minute 3 (p=0.0013), 0.0994mV lower at minute 4 (p=0.0266), and 0.0938mV lower at minute 5 (p=0.0362). Comparisons to baseline and between these minutes were insignificant. Based on these data, cervical dislocation appears to induce a greater stress response in laying hens.

Key Words: laying hen, euthanasia, electrocardiogram, cervical dislocation, CO2 displacement

12 Evaluation of two fill rates for CO2 euthanasia in turkeys.
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Carbon dioxide (CO2) is a conditionally approved euthanasia method for poultry. A maximum 40% CO2 gas concentration for poultry is recommended because high gas concentrations are potentially painful. However, there are no fill rate (FR, volume displacement/minute) recommendations for the use of CO2 in poultry. This study aimed to investigate the electroencephalographic (EEG) and behavioural responses of 21-day old turkeys subjected to either 21% (3 L/min, ‘Low’) or 84% (12 L/min, ‘High’) FR. Twenty four 21-day old turkeys were randomly assigned to each treatment and euthanized individually. Prior to euthanasia, baseline EEG was measured, and EEG, behaviour, and CO2 concentrations were recorded for 5-minutes following gas induction. EEG traces and video recordings were available for 8 birds in the Low FR and 11 in High FR. Data were analyzed using Proc Mixed procedure of SAS 9.4. Latency to gasping was shorter in High FR than in Low (9.2±1.2s vs. 14.2±1.4s, respectively; P≤0.02). Turkeys in High FR showed similar times for increases in delta waves (indicating insensibility) as in Low (44s and 56s; P>0.4), respectively. However, latency to loss of posture (behavioural indicator of insensibility) was shorter during High (37.4±4.6s) than Low FR (76.5±5.2s;
P \leq 0.0001). Delta waves were significantly suppressed (suggesting brain death) compared to baseline at 105s (P \leq 0.003) and 195s (P \leq 0.002) after High and Low FR, respectively. The results suggest that turkeys in both treatments were able to detect CO2 (less than 15s after gas induction) before it caused insensibility. High FR reduced the latencies to behavioural responses, time to insensibility, and death and resulted in shorter time between gas detection and insensibility.

Key Words: euthanasia, CO2, turkey, fill rate, humane killing

13 Are broiler chicks’substrate preferences altered by soiling or type of wood shavings? Valérie Monckton, Nienke van Staaveren, Alexandra Harlander, Animal Biosciences, University of Guelph, Guelph, Ontario, Canada.

Current farm housing systems keep broiler chickens in contact with their excreta throughout their lives. This soiled bedding can contribute to conditions that compromise the welfare of broiler chicks, such as wet litter and increased ammonia levels. To prevent this, farmers occasionally apply a chemical ammonia reductant to soiled litter or replenish litter with fresh bedding. Yet, it is unknown whether or not broiler chicks prefer to avoid soiled environments, or if they prefer any of these different litter management practices. Therefore, this study aimed to determine broiler chicks’ relative strength of preference for different litter management practices. 40 three-week-old broiler chicks were housed in six pens in a two-compartment consumer-demand test setup. Each pen had a ‘home’ (H) and ‘treatment’ (T) compartment that contained ad libitum feed and water. Dividing these compartments was a barrier with two one-way push-doors. We placed built up, soiled “home” shavings in the H compartment, while compartment T contained one of four litters: aspen wood shavings (FA), pine & spruce wood shavings (FP), soiled pine & spruce wood shavings (SP), or ammonia reductant treated soiled pine & spruce wood shavings (TSP). In addition to these four litters, we included a feed treatment (gold standard) that involved removing feed from H so that birds had to enter T to eat. The feed treatment was the only time feed was absent from any compartment. To evaluate the chicks’ motivation to access treatments, the push-doors leading to T weighed either 0% (raised doors), 10%, 20% or 30% of the chicks’ body weight. Generalized linear mixed models (GLIMMIX) assessed the effect of resource (feed, FP, FA, SP, TSP), door weight (0%, 10%, 20% and 30%), and their interaction on the maximum door weight pushed to enter T, time spent in T, and number of visits to T. Statistical significance was considered at P < 0.05. We found that chicks pushed a greater mean weight to access feed compared to the litters (P < 0.0001). As the door weight increased, we found that the birds spent more time in feed and less time in the litter treatments (P < 0.0001) and that chicks visited T less, though they visited feed more than the litters (P = 0.0048). Overall, the chicks worked more to access feed over all the litters and showed equal motivation for all the litters. Therefore, since chicks will not make decisions in the best interest of their health, caregivers should closely monitor litter conditions to reduce the incidence of health problems. Furthermore, the long-term effects of these practices on broiler chicks should be further investigated under commercial conditions.

Key Words: welfare, motivation, chicken, litter, preference

14 Spatial complexity during early life affects activity and navigation skills of laying hen pullets. Ana K. Rentsch, Tina Widowski, Animal Bioscience, University of Guelph, Guelph, Ontario, Canada.

Laying hen housing is shifting from caged housing to alternative housing systems. While allowing for more species-specific behaviours, the complexity of aviary housing can be challenging for hens. Increased risk of injury and inability to access resources can negatively affect laying hen welfare. Commercial rearing aviaries are intended to prepare pullets for the layer house, but system designs vary considerably. We hypothesise that pullets raised in aviary systems with greater spatial complexity and space during the brooding period will be more active in early life and develop better spatial navigation skills needed to navigate the adult aviary. Here we present data from the first of 4 replicates in an on-going study. 1400 Lohmann LSL-lite and 1400 Lohmann brown-lite pullets were raised in one of three styles of rearing-aviaries with increasing spatial complexity (aviary-style 1: least complex and style 3: most complex) or in conventional rearing cages (CC) until 17 weeks-of-age (WoA). Aviary pullets were enclosed in the system before given access to litter and multiple tiers at 6 WoA. Activity (defined as % time performing locomotory behaviours) was assessed by live-observation of focal-birds at 0, 2, and 4 WoA and at 6, 10 and 16 WoA. At 15 WoA, 15 hens from each treatment-group underwent a 5-task spatial-navigation-test assessing their ability to navigate 3-dimensional space. The pullets had to vertically navigate an experimental arena to access food. Tasks 1 to 4 involved upwards locomotion and the 5th a descending jump. Activity data were analysed with a generalised mixed effect model (GLMM). Success during the spatial navigation task was analysed in a binomial GLMM. A Tukey test with Bonferroni correction was applied for pairwise comparison after all models. In closed systems, there was an interaction between aviary-style and pullet strain (P = 0.007). Brown pullets from style 3 were more active than those in style 1 (z = 3.53, p = 0.005) and 2 (z = 3.51, p = 0.006) and compared to white pullets in style 2 (z = 3.33, p = 0.01) and 3 (z = 3.42, p = 0.008). In open systems, activity was affected by aviary-style (p = 0.004). Pullets from style 1 were less active than style 3 (z = 3.3, p = 0.003) and 2 (z = 2.4, p = 0.04). In the navigation task, pullets from CC were less likely to succeed than pullets from style 2 (z = 2.8, p = 0.02) and 3 (z = 2.7, p = 0.04). Preliminary results suggest that spatial complexity during the first 6 WoA affects brown feathered pullets more than whites. These effects seem to be long-lasting, affecting
spatial navigation skills until minimum 16 WoA and could impact laying hen welfare in aviary housing.

Key Words: pullet rearing, welfare, aviary housing, activity, spatial navigation


The broiler chicken has been genetically selected for a highly feed efficient, fast-growing phenotype with a large proportion of breast tissue. An inadvertent selection result is a top-heavy bird that spends an increasing amount of time inactive, causing leg disorders that negatively impact bird welfare. Environmental enrichment that stimulates exercise, a promising method to counteract lameness, is lacking in commercial broiler barns. Hence, a unique laser enrichment device was implemented to project two randomly moving red dots across the floor of two adjoining pens with the objective to motivate natural foraging behavior and ultimately, physical activity. Twelve-hundred Ross 308 broilers were housed in pens of 30 for 6 wk where half of the birds, considered laser-enriched, received enrichment treatment daily (four 4-min “laser periods”/day at 05:30, 11:30, 17:30, and 23:30) and the other half served as controls. Seven pens from each treatment (n=14) were video recorded d0-8 and once weekly wk 2-6. All birds in each video-recorded pen were categorized into two behaviors during 4-min laser periods, including percent of birds following the laser (in laser-enriched pens only) and percent of birds physically moving (whether following the laser or not), both calculated using the total number of birds in each pen. Data were analyzed using Proc Glimmix in SAS Version 9.4 (Cary, N.C.) with fixed effects of day/week, treatment, and day/week by treatment interaction. Within the laser-enriched pens only on d0-8, percent of birds actively following the laser was affected by day (P<0.0001), with a range of 1.4±0.30% of birds following on d8 up to 16.80±1.20% of birds following the laser on d0. Similarly, percent of birds following laser was affected by week (P<0.0001), with a range from 0.93±0.18% birds following on wk 6 up to 4.32±0.52% following on wk 2. When analyzing the total percent of birds moving in both laser-enriched and control pens d0-8, there was a significant treatment by day interaction (P<0.0001). The greatest difference in percent of birds moving during the laser periods was on d1, where 72.26±3.21% of birds moved in laser-enriched pens compared to 26.84±1.96% in control pens (45.42% difference). Weekly, the total percent of birds moving showed a treatment by day interaction (P<0.0001), with a range from 0.93±0.18% birds following on wk 2 up to 4.32±0.52% following on wk 6. The greatest difference in percent of birds moving was during the laser periods by day induction attempts (2±1) did not differ (P>0.1). SD did not affect latency to begin feeding (HD=274±10s; LD=288±10s; P=0.35) or other latencies (HD=288±10s; LD=274±10s; P=0.35) or other latencies (HD=288±10s; LD=274±10s; P=0.35). For TI, mean latency to righting (60±9s) or induction attempts (2±1) did not differ (P>0.1). Our preliminary findings suggest that broilers in enriched environments were more optimistic (shorter latency in JBT) and less anxious (shorter latency in ABT). However, the small sample size for JBT limits the ability to extrapolate findings. SD did not clearly affect broiler optimism.

Key Words: pullet rearing, welfare, aviary housing, activity, spatial navigation

16 Impact of environmental enrichment and stocking density on affective states of broiler chickens. Mallory G. AndersonGS*, Andrew M. Campbell, Alexa Johnson, Madi Casey, Leonie Jacobs, Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, United States.

Broiler chickens are often housed under barren conditions, which could negatively impact affective states. Environmental enrichment (EE) under low or high stocking density (SD) could improve affective states, yet we need data to support this theory. The objective was to evaluate optimism, anxiety, and fear in broilers with judgement bias (JBT), attention bias (ABT), and tonic immobility (TI) tests. In a 2*2 factorial, male broilers (n=1620; 12 pens) were housed in a highly-enriched (HE) or a non-enriched environment (NE) under high or low SD (high [HD]=41.2kg/m²; low [LD]=24.9kg/m² at d50). The multimodal JBT process included habituation, pre-training, training, and testing. Birds were trained to discriminate between a positively (mealworms) and negatively (nothing) reinforced cue that was black or white, placed either on the left or right of the arena. After training (n=36), 9 birds (25%) met the testing criterium (HE/HD n=2; HE/LD n=3; NE/HD n=3; NE/LD n=1). During testing, ambiguous cues of intermediate color and location were presented. Frequency (%) and latency to approach (s) ambiguous cues were measured. During ABT, anxiety (response to a perceived threat) was tested by playing an 8s conspecific alarm call and recording latency to begin and resume feeding (from a feeder with feed, oats, and mealworms), first vocalization, and first step (5 birds/pen; total n=60) on d30-33. The call was repeated if birds ate. TI assessed fear (response to a known threat) on d12 and d26 (3 birds/pen; total n=36). TI induction attempts (n) and latency to righting (s) were recorded. Data were analyzed with chi-square tests and mixed models in SAS. In the JBT, EE decreased latencies to approach cues (HE=33±3s, NE=46±4s; P=0.014), but did not affect frequency (P=0.83). HD birds tended to approach more often than LD birds (HD 65%, LD 48%; P=0.074), but latencies were similar (HD=36±3s, LD=44±4s; P=0.13). During ABT, EE resulted in shorter latencies to begin feeding after an alarm call (265±10s) compared to NE (296±10s; P=0.03). Other latencies did not differ (P>0.1). SD did not affect latency to begin feeding (HD=288±10s, LD=274±10s; P=0.35) or other latencies (P>0.1). For TI, mean latency to righting (60±9s) or induction attempts (2±1) did not differ (P>0.1). Our preliminary findings suggest that broilers in enriched environments were more optimistic (shorter latency in JBT) and less anxious (shorter latency in ABT). However, the small sample size for JBT limits the ability to extrapolate findings. SD did not clearly affect broiler optimism.
anxiousness, or fear. We recommend further study, yet our findings support the theory that enriched environments result in improved affective states in broiler chickens.

**Key Words:** Broiler chickens, Affective state, Judgement bias, Attention bias, Tonic immobility

17 Self- and conspecific-directed pecking behavior of commercial Pekin ducks. Yiru Dong*‡,#, Darrin M. Karcher, Marisa Erasmus, Department of Animal Sciences, Purdue University, West Lafayette, Indiana, United States.

This study investigated characteristics of ducks’ pecking behavior, including 1) the prevalence, frequency and duration of pecking behavior, 2) age-related changes in pecking behavior and preening; 2) body locations affected, and 3) occurrence of feather removal and feather eating. Behavior of two commercial flocks was video-recorded at 20-22d (P1), 27-29 d (P2), and 34-36 d (P3) and analyzed using scan (n=53,192 ducks/flock) (one observer) and focal (n=144 ducks/flock) animal sampling (inter-rater reliability: r=0.95). Proportions of ducks (scan sampling) performing pecking behavior (gentle feather pecking (GFP), severe feather pecking (SFP), aggressive pecking (AGGP) and self-picking (SELFP)) was recorded every 30 min (0900h to 1500h). The total number of ducks in the pre-selected viewing area (147–561 ducks/area) was counted at the start of the 30 min period to calculate the percentage of ducks performing each behavior. Randomly selected focal ducks were observed for 30 min (0945h to 1015h and 1345h to 1415h) to determine the duration (D) and frequency (F) of pecking behavior and preening, and the occurrence of feather removal and feather eating. Scan sampling data and focal behavioral durations were analyzed using PROC MIXED; behavioral frequencies were analyzed using PROC GLIMMIX (SAS 9.4). GFP, AGGP and SELFP were combined into one category, other pecking (OP), for analysis. Frequency and duration of GFP and OP were log (x + 1) transformed. Of ducks performing pecking behaviors, 90.9% performed GFP, 1.9% SFP, 6.8% AGGP; no SELFP was observed. Based on focal sampling, 83.3% performed GFP, 13.9% SFP, 16.7% AGGP, and 1.4% SELFP. Feather removal and feather eating were infrequent. The proportion of ducks performing GFP (Prop GFP; P<0.001), and the frequency (GFP; P<0.001; OP: P=0.04) and duration (GFP: P<0.001; OP: P=0.04) of GFP and OP were affected by age. GFP frequency, duration (back transformed means) and Prop GFP increased from P1 (F: 3.58, D: 6.14 s, Prop GFP: 0.840.06%) to P2 (F: 20.05, D: 84.94 s, Prop GFP: 1.190.06%, P<0.001), then declined from P2 to P3 (F: 5.96, D: 12.74 s, Prop GFP: 0.830.06%, P<0.001). Preening frequency and duration changed with age (P<0.001). OP frequency (P=0.03) and duration (P = 0.03) were higher in P2 (F: 0.77, D: 1.15 s) vs. P1 (F: 0.25, D: 0.31 s). Body locations affected by GFP included the tail, wings and back. In conclusion, GFP between conspecifics was the most frequently observed behavior. Age was a major factor affecting the development of pecking behavior, which peaked at P2 and was most frequently directed at the tail, wings and back, coinciding with feather development and growth in these locations.

**Key Words:** Pekin duck, feather pecking, feather picking, pecking behavior, welfare

18 Similarity in movement patterns in chickens increases with time and social association. Klara J. Grethen*‡, Yamenah Gómez1, John Berezovski2, Yandy Abreu Jorge1, Sabine Gebhardt Henrich1, Sabine Vögeli3, Ariane Stratmann1, Michael J. Toscano0, Bernhard Völkl1, ‡Research Center for Proper Housing: Poultry and Rabbits (ZTHZ), Division of Animal Welfare, VPH Institute, University of Bern, Bern, Switzerland, 1Department of Clinical Research and Public Health, VPH Institute, University of Bern, Bern, Switzerland, 3National Centre for Animal and Plant Health, San José de las Lajas, Cuba, 3Animal welfare and 3Rs, Division of Animal Welfare, VPH Institute, University of Bern, Bern, Switzerland.

Under commercial conditions, flock sizes of hens often exceed group sizes that would naturally occur in the ancestral Red Jungle fowl. Although social organisation within groups affects the decisions of an individual, and consequently access to resources and group movement, the impact of social organisation in these large groups on the daily activities of individuals is scarcely documented. Therefore, we used a total of 421 laying hens (Brown Nick) as focus animals across 4 pens, containing 355 hens per pen, with free-range access, to examine consistency of movement patterns and social associations within each pen. The movements represented transitions between three types of external areas (wintergarden, stone yard, free-range) and the barn, collected over 72 days via a RFID tracking system. Analyses were conducted utilising a combination of social network analysis on co-occurring transitions between areas and Dynamic Rime Warping based on daily spatio-temporal movement patterns. The Dynamic Time Warping method estimates similarities between time series allowing identification of clusters similar in movement. We found consistent daily movement patterns of transitions between pen areas for individuals, with clusters of animals sharing similar movement patterns. Moreover, many birds appeared to associate preferentially with a stable cluster of individuals resulting in what appeared as several communities within the larger flock. Further investigation revealed a correlation between the similarity of movement patterns and social associations, which strengthened over time. These results highlight how social organisation can have a decisive role in shaping and/or enhancing individual movement variation within a flock despite low genetic diversity and a homogenous environment. Furthermore, the study demonstrates subgroup dynamics suggesting a specialisation based on area usage within large ranging flocks. We believe the described techniques represent an innovative means to understand how animals behave within large groups and offer potential to explain variations in production and health at both the individual and flock levels.
**Key Words:** social network, phenotype variation, individuality, movement tracking, behavioural clustering

19 **What do farmers think about health and welfare issues in turkey production?** Nienke van Staaveren*, 1, 2, Emily M. Leishman1, Sarah Adams1, Ben J. Wood1, 1, 4, Alexandra Harlander2, Christine F. Baes1, 1, 4, ‘Centre for the Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, 1The Campbell Centre for the Study of Animal Welfare, Department of Animal 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.

Farmers are responsible for decisions in the day-to-day management and are the primary caretakers of animals. Thus, they play a vital role in ensuring animal health and welfare and it is important to understand how they perceive these issues. A cross-sectional survey among turkey farmers in Canada was conducted to provide insights into the main perceived reasons for culling and mortality in turkeys. Additionally, farmers were asked to indicate how much of an issue they considered different health and welfare-related traits (i.e., mortality, aggressive pecking, disease, leg injuries, leg deformities, breast injuries, and varying body size) on a 3-point scale for their own farm and for the sector as a whole. A final 83 responses were included (20% response rate) and multinomial models were created to determine differences in farmers’ ratings between their farm and the sector. Leg-related issues (90.0%), sickness (60.5%), and small body size (58.0%) were the most frequently mentioned perceived reasons for culling of birds. Perceived reasons for mortality were often unknown (59.7%), or they were attributed to cannibalism (41.6%) or dehydration (42.9%). In comparison to their own farm, farmers perceived disease (OR = 4.9, 95%CI: 2.33 – 9.71), leg deformities (OR = 2.7, 95%CI: 1.35 – 5.41), and breast injuries (OR = 4.9, 95%CI: 2.42 – 9.78) to be more of an issue for the turkey production sector as a whole. These findings contribute to our understanding of what kind of health and welfare-related issues turkey farmers struggle with and how they perceive these. This can help identify priorities that could be targeted through research, changes in management or breeding approaches, ultimately improving both the well-being of farmers and turkeys.

**Key Words:** Turkey, Farmer, Attitude, Mortality, Welfare


An increase in public interest led poultry producers to focus on improving animal welfare standards. Two major focal points for broiler chicken welfare improvements are reducing stocking density and increasing environmental complexity. Therefore, this study’s objective was to determine the impact of stocking density and environmental enrichment on broiler chicken performance. A 2x2 factorial approach was tested with stocking density and environmental enrichment as factors. Treatments were high stocking density [HD] (40-42kg/m²), low stocking density [LD] (20-22kg/m²), and pens containing highly enriched environments [HE] or no additional enrichment [NE]. Highly enriched environments included functional spaces, toys, and novel objects. Male broilers (n=1620) were housed in 12 14m²-pens with one of four treatments (HD/HE, LD/HE, HD/NE, LD/NE). Performance data were collected at the end of the starter (d15), grower (d29) and finisher phase (d50). Birds and feed were weighed to assess mean body weight (BW), feed conversion ratio (FCR), and mean feed intake per bird (FI). Data were analyzed in SAS using mixed models. Results are presented as LSmeans±SEM. When comparing treatment groups, birds in LD treatments performed better during the starter (d15 BW=511±7g; FI=670±0.8g), grower (d29 BW=1720±12g; FI=1820±13g), and finisher phase (d50 BW=4.42±0.1kg; FI=4.51±0.05kg) than birds in HD treatments (d15 BW=502±7g; FI=590±8g, d29 BW=1710±12g; FI=1775±13g, d50 BW=3.99±0.1kg; FI=4.31±0.05kg). Similarly, d0-50 FCR was best for LD (1.572 FCR) compared to HD (1.674). HD negatively affected d50 BW (P=0.028), but did not affect d15 or d29 BW (P>0.1). HD negatively affected FI (starter P<0.001; grower P=0.031) compared to LD, and tended to negatively affect d0-50 FCR (P=0.055). Environmental complexity did not affect performance data (P>0.1), except in an interaction with density for finisher FI (P=0.046). Finisher FI was lower (0.3kg) for HE/HD birds compared to HE/LD and LE/LD (pairwise P<0.01), and tended to be lower than LE/HD. In line with previous research, our results show the beneficial effects of low stocking density on broiler chicken performance. Environmental complexity did not negatively or positively impact performance, with the exception of the last phase when feed intake decreased under high stocking density compared to birds in the other treatment groups. To conclude, environmental enrichment could be integrated into broiler chicken production systems without impacting bird performance. Our ongoing research evaluates the impact of these factors on welfare parameters.

**Key Words:** Stocking Density, Environmental Complexity, Performance, Broiler, enrichment

21 **Attracting young turkey poults to feeders to avoid litter-eating.** Sameeha Jhetam*, 5, Kailyn Beaulac, Karen Schwean-Lardner, *Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Litter-eating in turkey poults is a welfare and production concern as it may lead to impaction of the digestive tract,
resulting in starvation and/or mortality. The most common litter substrate reported to cause mortality in poults from ingestion is wood shavings (WS). Feed-based attractants may urge poults to consume feed rather than WS. The objective of this study was to identify an effective feed-based enrichment (feed dyed green (GF) or red (RF), LED lights on feeders (LED), red colored paper top-dressed with feed as a supplemental feeder (P), or control (C)) that will encourage feed consumption and deter litter-eating during brooding. Nicholas Select turkey hens (n=620) were placed in pens (31 poults/pen) with 4 replications per treatment (trt) and were brooded in brooder rings on WS. Birds were provided a metal tube feeder (MF), bell drinker, and a supplemental feeder (SF) and drinker. Live behaviour observations were performed for 10 minutes at 1-minute intervals for each pen twice a day at day 1, 4, 7 and 10. Behaviours recorded included feeding at the MF and SF, drinking, and litter pecking. Group BW was measured at day 0 and 11 to determine average BW. On day 5, 2 birds/pen were culled, and contents of the gizzard were removed to determine percent of WS in the gizzard on a dry matter basis. Samples were dried for 24 hrs, then rinsed in a sieve to dissolve feed particles. WS were separated, then dried for 24 hr before weighing. Data were analysed as a complete randomized design via ANOVA (Proc Mixed of SAS 9.4). Differences were significant when $P \leq 0.05$ and trends were noted when $P \leq 0.10$. On day 1, there was a tendency ($P=0.07$) for birds to spend a greater percent of time feeding at the MF with LED lights (2.26%) compared to C (0.16%), RF (0.48%), GF (0.20) or P (0.12%). There was a higher percent of birds feeding at the SF in P compared to LED ($P=0.03$) on day 1, with LED birds feeding the least at the SF. Time at the SF on day 4 was higher for P compared to LED or RF ($P<0.01$). Trt did not impact percent of time spent drinking or litter pecking at all ages, or for feeding at the MF or SF at day 7 and 10. Trt did not impact day 11 BW (311, 309, 301, 320, and 314g for trt C, RF, GF, LED, and P, respectively). The percent of WS (dry matter basis) in the gizzard contents was highest in C (58.44%) compared to RF (28.21%) ($P<0.01$) with no differences between other trt. The results indicate that feed-based attractants may reduce litter-eating, with feed dyed red being the most effective.

**Key Words:** coloured feed, paper, feed-based enrichment, brooding, LED Lights

22 The impact of light intensity and strain on behavior and welfare of egg-strain pullets reared in perchy systems from 0 to 16 weeks of age. Jo Ann Chew,1, Tony Shynkaruk1, Eugenia Herwig2, Tina Widowski3, Karen Schwean-Lardner1,1 Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada; 2Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada.

The objective of this study was to determine the impact of light intensity (L) and strain (S) on pullet behavior and welfare. Three L (10, 30, or 50 lux, provided by white LED lights) and two Lohmann S (Brown-Lite (LB) and LSL-Lite (LW)) were used in a 3 × 2 factorial arrangement in a randomized complete block design. LB and LW (n=1,800 per S) pullets were floor reared in pens (3 of each S per room; not visually exclusive) within light tight rooms (4 per L) from 0 to 16 wk of age. Each pen contained a system of four parallel perches (0.3 m apart, 0.6 m high), ramp, drinker line, and two tube feeders. At 15 wk of age, pullet fear and stress levels were assessed with a novel object test (8 replicate pens per L × S) and blood heterophil/lymphocyte (H/L) ratios (12 replicate pens per L × S), respectively. Pullets (4 replicate pens per L × S) were video recorded (24 h) at 4, 8, 13, and 16 wk. Behavior was analyzed via instantaneous scan sampling (20 min intervals during light periods; 144 scans per L × S at 4 wk, 96 scans per L × S at 8, 13, and 16 wk) and continuous sampling for 24 h for successful and failed jumps between resources in the pen. The effect of L, S, and their interactions were analyzed using Proc Mixed (SAS 9.4) with Tukey’s range test to separate means. Differences were significant when $P<0.05$. L did not affect fear or H/L. LB pullets had a longer latency to peck at the novel object (676 vs 212 s, $P<0.05$) and higher H/L (0.26 vs 0.13, $P<0.05$) than LW. L did not affect pullet behavior until 13 and 16 wk; 50 lux pullets performed more comfort (preening, stretching, feather ruffling, dustbathing) behaviors than those in 10 lux ($P<0.05$), whereas 10 lux pullets pecked at walls more than those in 50 lux ($P<0.05$). At all recorded ages, LW pullets performed more resting and comfort behaviors, while LB expressed more exploratory (gentle pecking, litter or environmental pecking) behaviors ($P<0.05$). LW were also more active (standing, walking, jumping) at 4 wk and more nutritive (feeding, drinking) at 4 and 16 wk than LB. L did not affect jumps, however S did. At all observation periods, LW pullets performed more successful and unsuccessful jumps between resources in the environment than LB pullets ($P<0.05$). However, success percentages of jumps did not differ between S except at 16 wk; LB pullets had statistically (but numerically small) higher success percentage than LW (99.93% vs 99.88%, $P<0.05$). Across all weeks, all birds had an average of 99.65% success rate at jumps within the environment. The results suggest that L between 10 and 50 lux does not impact pullet fear, stress response, or space use, however, it may affect bird behavior. Conversely, S influences all of these factors.

**Key Words:** Lohmann Brown-Lite, Lohmann LSL-Lite, novel object test, heterophil/lymphocyte ratio, environmental navigation

23 Rearing enrichments, outdoor ranging and environmental stress impacted free-range hen welfare. Md Saiful Bari,1,2, Jeff A. Downing3, Tim R. Dyall3, Caroline Lee2, Dana L. Campbell2,1 School of Environmental and Rural Science, University of New England, Armidale, New South Wales, Australia; 3Agriculture and Food, Commonwealth Scientific and
Rearing enrichments may contribute to better adaptation of hens as they move from indoor rearing to a free-range system and thus may improve welfare. Individual variation in outdoor ranging may also contribute to differences in welfare. We assessed the effect of rearing enrichments and ranging on hen welfare along with the effect of an imposed stressor on hen welfare and egg quality. Hy-Line Brown® chicks (n = 1386) were reared indoors until 16 weeks with 3 enrichment treatments including a 'control' group having no extra materials over standard floor litter, a ‘novelty’ group providing novel objects (e.g. balls, bottles, bricks, brooms, brushes, buckets) that changed weekly, and a ‘structural’ group with H-shaped perching structures. At 16 weeks of age, the pullets were moved to a free-range system with three replicates of each rearing treatment. All hens were leg-banded with microchips and daily ranging was assessed from 25 to 64 weeks via radio-frequency identification technology. Individual hen welfare assessments were performed at 27, 33, 43, 56 and 64 weeks of age and correlated with their outdoor ranging time prior to these assessment dates. An environmental stressor was applied by reducing the range area by 80%. Changes in ranging behaviour, egg quality parameters and the albumen corticosterone concentrations were evaluated. GLMMs showed significant interactions between hen age and rearing treatment on live weight, the number of comb wounds, plumage coverage and toenail length (P < 0.006) with the enriched hens showing more consistent live weight, fewer comb wounds and better plumage coverage, whereas the structural hens had shorter toenails. Plumage coverage showed a positive relationship with range use across all age points (P < 0.0001). The average number of visits outside significantly increased following the imposed stressor with a higher increase in the number of visits in the structural group of hens (P = 0.03). There were significant interactions between rearing treatment and stressor treatment for albumen corticosterone concentrations with the structural hens showing a decrease in concentrations immediately post-stress, but the control and novelty groups showed an increase (P < 0.006). The stressor treatment affected most egg quality parameters across all rearing treatment groups (all P ≤ 0.004) with some parameters showing an increase in value and others a decrease. Rearing treatments and ranging affected hen welfare across the flock cycle. Egg quality parameters were impacted by stressors, but rearing enrichments did not minimise these effects. Overall, it appears that rearing enrichments along with optimum ranging thus might be recommended for positive effects on hen welfare.

Key Words: novel objects, range access, plumage coverage, corticosterone, RFID

Comparison of footpad dermatitis scores in market turkey hens. Gabriella Furo, Carol Cardona, Yuzhi Li, Sally L. Noll, Department of Animal Science, University of Minnesota, Saint Paul, Minnesota, United States, Department of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, Minnesota, United States, West Central Research and Outreach Center, University of Minnesota, Morris, Minnesota, United States.

Footpad dermatitis is a welfare concern characterized by skin inflammation with erosion and ulceration of the footpad. Assessment at processing allows better observation. Live bird scoring (LS) is difficult due to adherence of litter but could yield important information. The study objective was to investigate agreement between LS and postmortem (PM) score. Hybrid Converter hen turkeys were housed at different stocking densities and bedding material. The footpads of 6 and 14 wk old hens (N=24 per age) were scored by two observers after minimal removal of material (LS) and then re-scored PM after sampling and storage in 10% buffered formalin resulting in a cleaner pad. Footpads were scored (Berg, 1998 no lesion and 2- severe) and a composite score given. One sample proportion test was used to test the agreement of LS and PM scores of the same turkey hen and age group. Spearman correlation tested the association of LS and PM scores. At 6 wk, average LS was 0.17 with 83% turkeys having no lesions and 17% score of 1. For PM, 96% of hens scored 0 and 4% scored 1 with an average of 0.04. The sample proportion test indicated 83% agreement of LS and PM scores with 95% CI (62%, 95%). The agreement was high due to the majority of the turkeys having no lesions. At 14 wk, 13, 63, and 25% of turkeys had LS score of 0, 1, and 2, respectively with average of 1.13. The average PM score was 1.13 with 33, 21, and 46% having scores of 0, 1, and 2, respectively. A moderate correlation was found between LS and PM (r=0.59, P=0.0028) and reported earlier (Furo et al., 2017). Agreement was 54% (95% CI 33, 74%). The most discrepancy in score occurred at score 1. For hens at 14 wk with a LS of 1 (N=15), three were subsequently re-scored PM as 0 and seven as 2. In conclusion, agreement between LS and PM footpad score was better for turkeys assessed at 6 wk versus 14 wk. At 14 wk, the LS and PM discrepancy at score 1 (mild lesions) indicated it was more difficult to assess perhaps due observation conditions. Thus further data collection is needed, with possible revision of current footpad scoring schemes advised. Funding provided by USDA NIFA no. 2016-67015-24457.

Key Words: footpad dermatitis, assessment, turkey, postmortem, animal welfare

Prevalence and severity of footpad dermatitis in commercial turkeys flocks in the Midwestern United States. Gabriella Furo1, Darrin M. Karcher1, Kailynn Scoles2, Shawna L. Weimer3, Sally L. Noll1, Department of Animal Science, University of Minnesota, Saint Paul, Minnesota, United States, Department of Animal Sciences, Purdue University, West Lafayette, Indiana, United States, Animal and Avian Sciences, University of Maryland, United States, Animal Science, University of Minnesota, Saint Paul, Minnesota, United States, Department of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, Minnesota, United States, West Central Research and Outreach Center, University of Minnesota, Morris, Minnesota, United States.
Footpad dermatitis (FPD) in turkeys is a significant welfare and economical problem worldwide caused by high litter moisture, however little is known about the prevalence of this condition in commercial flocks in the Midwestern United States, where significant turkey production occurs. The objective was to examine the prevalence and severity of FPD in commercial turkey flocks in conventional housing. A total of 18 turkey flocks (female N=8, male N=10) located in Indiana and Minnesota were visited twice: first around the end of brooding period (4-7 wk of age), then before market (females 15-17 wk, males 16-19 wk). Data were collected between March 2017 and May 2018, the majority of poult flocks were visited in September 2017 (N=12), and market flocks evaluated in December, 2017 (N=12). At each visit, both footpads of 25-100 of randomly selected turkeys were cleaned gently, and footpad scores (FS) were assigned on a 3 point scale by Berg (2015) (0- no lesions, 1- mild and/or superficial lesions, 2- severe and/or deep lesions, FS0, FS1 and FS2, respectively), then the worst FS was recorded. The footpad data were explored by descriptive statistics using R, and 95% confidence interval (CI) was calculated (Stata). In brood, at first sampling, and considering the entire dataset (N=1739), the majority of turkey poult s (73%, CI 72-75%) had no footpad lesions (FS0), while 24% and 3% received FS1 and FS2, respectively. Within female poult s (N=800), 50% (CI 47-53%), 43% (CI 40-46%) and 7% (CI 5-9%) of the turkeys received FS0, FS1, and FS2, respectively. The majority (88%, CI 86-90%) of the male poult s (N=939) had no FPD lesions (FS0) while 12% (CI 10-14%) and 1% (CI 0-1%) received FS1 and 2, respectively. At the second sampling, the market-weight turkeys (N=1670) had 7.5% (CI 6-9%), 53% (CI 50-55%) and 40% (CI 37.5-42%) footpad scores of 0, 1 and 2, respectively. Of the market-weight hens (N=800), 6% (CI 5-8%) had no footpad lesions (FS0), while FS1 and FS2 prevalence was the same (47%, CI 43-50%). Market-weight males (N=870) had 9% (CI 7-11), 56% (CI 52-59%), and 35% (32-39%) incidence of 0, 1, and 2 footpad scores, respectively. In conclusion, footpads without any lesions (FS0) were observed with much higher frequency at the end of brood as compared to finishing. Females exhibited more severe lesions (FS2) compared to males, especially at the end of brooding. A possible explanation for this difference between gender could be related to BW or to different management conditions, such as stocking density which could influence moisture content of the litter. The project was funded by USDA NIFA no. 2016-67015-24457.

Key Words: footpad dermatitis, turkey, prevalence, severity, animal welfare

26 Use of Environmental Enrichments in colony caged Japanese Quail to reduce damaging behaviors. Melissa Rudo, Chirantana Mathkar¹³*¹°C, Rachel Dennis, ANSC, University of Maryland, College Park, Maryland, United States. Japanese quail (Coturnix japonica) breeding colonies are often maintained in barren environments. Those facilities that utilize environmental enrichments (EE) generally use stimulatory enrichments such as foraging mats and exploratory items, such as beads, to redirect pecking and increase the complexity of the environment. However, aggression and mating behaviors continue in these environments. We designed this study to evaluate the effectiveness of different EEs on the well-being of breeding quail. Utilizing a latin square design we provided each of 12 breeding colony cage of quail (2 males and 4 females per cage) with a mirror, cuttlebone, foraging mat, protective hut, or no EE for two weeks each. Behaviors were recorded for 4 hours per day, 3 days per week and analyzed by scan sample (one scan per minute). Eggs were collected daily and incubated, hatchability numbers were analyzed by treatment. All EEs reduced walking compared to a barren environment (P=0.001) but did not affect overall inactivity levels. Protective huts, mirrors and foraging mats reduced severe feather pecking and aggressive pecking (P=0.01 and P=0.05). Mountings were reduced only in cages with protective huts (P=0.009), but did not alter fertility or hatchability of eggs. Standing decreased in birds provided with mats and mirrors (P=0.02), while sitting was increased in these birds (P=0.04). Vigilance was increased when mirrors were first introduced to colony cages (P=0.02), however, after 2 wks of acclimation to the mirror, vigilance was greatly reduced compared with control birds (P=0.01). These data suggest a great benefit of appropriate EEs in breeding colonies of quail. We have also shown that protective EEs such as our protective huts, can be a great benefit to reduce excessive mountings.

Key Words: Quail, Environmental Enrichment, Welfare, housing, behavior

27 Are beak growth and pullet performance affected by natural beak smoothing feeders and outdoor vegetation density? Xavier Averós*¹, Paul Patterson¹, Nuket Acar¹, Iván Pineda¹, Imna Estevez², ³, Animal Production, Neiker, Arauke, Spain, ²Penn State University, University Park, Pennsylvania, United States, ³Universidad Nacional Autónoma de México, Ciudad de México, Mexico, ¹ikerbasque Basque Foundation for Science, Bilbao, Spain.

Current trends in animal welfare include the reevaluation of beak trimming as a husbandry practice. This results in a new challenge for the industry as no safe alternatives exist to manage untrimmed laying hens. Natural Beak Smoothing feeders (NBS, Roxell) effectively control feather pecking in broiler breeders and increase industry profit. NBS feeders can also be used in egg laying strains. Pullet welfare with outdoor access also depends on vegetation, as it provides weather and predator protection. This study tested the viability of NBS compared to standard feeders (CON) for growth, beak parameters and feather damage in pullets with outdoor access. Day-old ISA Brown pullets (558) were randomly assigned to 3 NBS and 3 CON pens for brooding.

Key Words: beak growth, pullet performance, natural beak smoothing feeders, outdoor vegetation density, animal welfare, housing, welfare.
At 2 wks of age ISA Brown pullets (496) were divided into 16 pens of 31 pullets (8 NBS, 8 CON pens). From 8 wks all birds had access to 125 m² outdoor parks with LOW or HIGH vegetation density in a 2×2 factorial design. LOW parks had 9 trees and shrubs, with 2 side-rows of 3 willows and a central row with a linden, cypress, and apple tree. HIGH parks had 27 trees and shrubs, with 2 side-rows of 9 willows and a central row with 3 linden, cypress, and apple trees. All birds were weighed, mean body weight (BW) coefficient of variation (CV) calculated, and feather condition assessed during even wks until 16 wks of age. Beak scores from 10 birds/pen were also assessed. Beak length was: 0=upper and lower mandible of equal length, 1=upper mandible slightly longer and 2=upper mandible notably longer. Beak sharpness was: 0=smooth beak tip, 1=mildly sharp, and 2=sharp. Effects of feeder type, vegetation density, wk and significant 2-way interactions on BW and CV were tested using repeated measures ANOVA. Effects of feeder type, vegetation density, wk and significant 2-way interactions on beak sharpness and length were tested using an ordered multinomial analysis. At 12 and 14 wks BW was significantly higher for CON compared to NBS pullets (1,180 vs 1,139g; P=0.0004, and 1,275 vs 1,243g; P=0.0205, respectively). A similar trend was observed at 16 wks (1,431 vs 1,401g; P=0.0579). At 4 wks BW CV was highest (9.81%), and lowest at 16 wks (6.52%; P=0.0001). Pullets in NBS had significantly shorter and less sharp beaks than CON (odds ratios 0.348; P<0.0001, and 0.299; P=0.0050 respectively). Feather damage was observed from 14 wks in a few pens and at 16 wks, was found in 3 CON pens (3.23, 25.81 and 61.29% incidence) and 2 NBS pens (3.23 and 19.35% incidence). No mortality was observed during the experiment. Vegetation density showed no effect. Findings suggest the NBS feeder can promote the natural smoothing of pellet beak length and sharpness with a minimal impact on growth.

Key Words: Natural beak smoothing, Performance, Pullet, Vegetation density, Welfare

28 Impact of Vegetation Density on Outdoor Paddock Utilization and Behavior by Growing Pullets.

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Outdoor paddocks in poultry production are often underutilized, which represents a sub-optimal use of resources. One factor likely contributing to this is vegetation density, as it may be expected that a higher density is perceived by birds as more protective towards weather and predators, promoting greater use of outdoor areas. Therefore, a pullet study aimed at determining the use of outdoor space and the influence of vegetation density was undertaken along with a co-factor measuring the impact of Natural Beak Smoothing feeders (NBS, Roxell), compared to standard feeders (CON) in a factorial design. At 2 wks of age ISA Brown pullets (496) were divided into 16 pens (8 NBS and 8 CON), with 31 pullets per pen. From 8 wks of age birds had access to 5 × 25 m outdoor paddocks, with 2 vegetation densities. Low density paddocks consisted in 9 trees & shrubs distributed in 3 rows with 2 side-rows of 3 willows each and a central row with a linden, cypress and apple tree. High density paddocks (3-fold greater) consisted of 27 trees & shrubs distributed in 3 rows with 2 side-rows of 9 willows each and a central row with 3 linden, cypress and apple trees. Pullets were allowed access to the paddock treatments 5 d/wk from 8:00 to 16:00 hr via automatic pop-hole doors. Between 8 and 16 wks of age, the outdoor pullets were observed twice/wk by 2 observers after pop-holes were open. Each observation day the individual pullet ID number, her X-Y location coordinates and behavior were recorded during the 5-min observation period and transferred to the Chickitzz software. The percentage of pullets observed outdoors and their distance from the pop-hole (cm) were then calculated. The mean percentage of pullets utilizing the outdoor paddock was higher during wk 15 (53.2 ± 3.3 %) compared to wk 8 (41.1 ± 1.9 %; P < 0.0001), and highest overall during wk 14 (66.1 ± 3.9 %). Mean pullet distance from the pop-hole increased between 8 and 15 wks from 283 ± 7 to 409 ± 12 cm, respectively (P < 0.0001). Although vegetation density was expected to promote greater use and penetration of the rectangular paddocks, no effect of vegetation density was observed. This may be because all trees & shrubs were young and foliage density was not great enough between the treatments to distinguish a preference for cover or protection by the pullets.

Key Words: Pullet, vegetation, density, behavior, performance

29 Effect of rearing aviary style and genetic strain on musculoskeletal characteristics of layer pullets. Amanda Pufall*, Tina Widowski, Animal Biosciences, University of Guelph, Guelph, Ontario, Canada.

Various factors can affect the musculoskeletal development of pullets, including rearing environment. Pullets reared in aviaries have been found to have stronger bones and larger muscles compared to those reared in conventional cages, presumably due to increased locomotion and load-bearing exercise in the aviary system. However, commercial rearing aviaries vary in design, and offer different opportunities for locomotion, particularly during the brooding period. This study compared the musculoskeletal development of brown and white strains of pullets in three styles of rearing aviaries on commercial farms: Style 1 (S1) chicks start in brooding compartments outfitted with two low perches; Style 2 (S2) chicks start in brooding compartments with an elevated platform and three perches of various heights; Style 3 (S3) started chicks in an open concept, multi-level system that spanned the length of the barn. In all styles, producers opened the systems after a few weeks providing pullets...
access to a litter floor and multiple tiers. Fifteen commercial pullet flocks located in Ontario and Alberta, Canada (5 flocks per style; 7 white and 8 brown) were visited twice: at 68.5 ± 4.94 (V1) and 111.5 ± 3.45 (V2) days of age. At each visit, 8 pullets per flock were randomly selected and euthanized for dissection. The breast, bicep, and leg muscles, and the long bones of the wing and leg were removed to compare muscle weights and bone-breaking strength (BBS). All variables were corrected for BW. A generalized linear mixed model was used to assess the effects of visit, strain, aviary style, and their interactions. Aviary style affected the strength of the leg bones; both tibia (P=0.0162) and femur (P=0.0004) were stronger in S3 than S1, with S2 being intermediate. There was a style x strain interaction for pectoralis minor weight; white strains in S2 had the heaviest and brown strains in S2 had the lightest pectoralis minor (P=0.0104). White strains had heavier pectoralis major (P<0.0001), lighter leg muscles (P<0.0001), and stronger femurs (P<0.0001) than brown strains. Tibia strength and bicep weight both had a visit x strain interaction. Tibial BBS of both strain colours were the same at V1 and decreased over time. At V2, white strains had stronger tibias than brown strains (P<0.0001). Similarly, bicep weight did not differ between strains at V1 (P>0.05), but brown strains had heavier biceps than white at V2 (P=0.0153). Both aviary style and strain affected the musculoskeletal development of pullets. These developmental differences may affect the long term skeletal health and well-being of laying hens housed in non-cage systems.

Key Words: Pullet, Rearing, Aviary, Musculoskeletal development

30 Comparison of in vivo and ex vivo three-dimensional keel bone models at two timepoints in brown laying hens. Kaylee Montney, Prafulla Regmi, Cara Robison, Brittny Emmert, Darrin M. Karcher, 1Department of Animal Science, Michigan State University, East Lansing, Michigan, United States, 2Department of Animal Sciences, Purdue University, West Lafayette, Indiana, United States, 3Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, United States.

Accurate quantification of bone health is essential for proper assessment of skeletal health in laying hens. Computed tomography (CT) provides accurate measurement for bone parameters in live birds through three-dimensional (3-D) model analysis allowing for longitudinal studies to be conducted. Valuable information may also be gained from digital models of bones scanned ex vivo, particularly when in vivo scans are not feasible. Ex vivo digital twin, digital replica of the physical keel bone, may not be equivalent to in vivo due to bird movement during scan and human handling prior to or post scan. The purpose of this study was to determine differences between in vivo and ex vivo digital twins. Lohman brown birds were housed in either multi-tier aviary systems (AC) or in litter-and-slat floor pens (FC). All live 24-wk FC (N=30) and 52-wk AC birds (N=125) were CT scanned in restraint limiting devices within a four-hour block of time. Within two hours of return to laying hen facility all birds were humanely euthanized by cervical dislocation and had keel bones and breast tissue removed according to standard procedures by trained personnel. All keels were sealed in plastic bags and frozen, then thawed at time of ex vivo scan. CT images were reconstructed and 3-D models of the keels were generated using Materialise software (Plymouth, MI, USA). A triangular mesh was overlaid on each keel and in vivo keel images were superimposed onto ex vivo images. For normalization, the cranial portion of each bone was removed. The total mm difference between each keel’s in vivo and corresponding ex vivo mesh points was calculated using the part comparison tool in 3-Matic software. Each keel was scored based on sum mm difference (A < 2000 mm, 2000 mm ≤ B ≤ 4000 mm, C > 4000 mm) which was calculated using the MEANS procedure in SAS 9.4. The main effects of state (alive or dead), hen age, and score were evaluated using the MIXED procedure in SAS 9.4. The 24-wk FC hens were 73.33% A, 16.67% B, and 10.0% C, while 52-wk AC hens were 59.20% A, 36.80% B, and 4.0% C. A significant difference between 24 and 52-wk average mm difference (P=0.004) and sum mm difference (P=0.001) was found. At the 95th percentile differences between 24 and 52-wk B scores (P=0.01) and 24 and 52-wk C scores (P=0.001) revealed that bird age plays a role in the susceptibility of post-mortem keel damage. Results show that approximately 62% of ex vivo models are equivalent to in vivo and 33% show additional minor damage to the keel bone prior to ex vivo scan. Post CT scan handling and cervical dislocation resulted in additional damage in 5% of birds suggesting keel bone damage in those birds would have been overestimated when only examining ex vivo keels.

Key Words: computed tomography, laying hen, keel bone, 3-D model, digital twin

31 Using accelerometers to examine differences in inactivity between conventional and slower growing broiler chickens. Lauren Dawson, Zhenzhen Liu, Tina Widowski, Stephanie Torrey, Animal Biosciences, University of Guelph, Guelph, Ontario, Canada.

Selection for rapid muscle growth has led to larger, more efficient broiler chickens; however, conventional broiler chickens (CONV) experience health and welfare issues, which may cause or be caused by higher levels of inactivity. As such, there has been increasing interest in using slower growing broiler strains (SLOW) for commercial production. As part of a large-scale study, our objective was to compare the inactivity levels of 14 broiler strains (2 CONV, ADG30-47 = 66-69 g/day; 12 SLOW, ADG30-47 = 44-56 g/day). Each strain, raised under similar conditions (30 kg/m²), was assessed over 3 replicates, in 4 pens per replicate in a randomised incomplete block design. One male and one female per pen (n = 153 pens; 138 males, 142 females; 14-24 birds/strain) were outfitted with omni-directional accelerometers (Actical®; frequency 0.035-3.5 Hz, range
0.05-2 G) to continuously monitor activity from day 22 to 46; accelerometers were previously validated through video analysis. The number of 15-second epochs with no activity were summed per day per bird and analysed using a repeated measures generalised linear mixed model. For analyses, SLOW strains were further categorised as fast SLOW (F-SLOW, $ADG_{0-61} = 54-56$ g/day), moderate SLOW (M-SLOW, $ADG_{0-61} = 50-51$ g/day) and slow SLOW (S-SLOW, $ADG_{0-61} = 44-48$ g/day). Overall, CONV birds were inactive for $18.4 \pm 0.6$ h per day, whereas SLOW birds were inactive for $17.4 \pm 0.6$ h per day ($p = 0.07$). Inactivity increased with age ($p < 0.01$), and females were more inactive than males ($p < 0.01$). At the same age, CONV chickens were more inactive than S-SLOW chickens until day 35 ($p < 0.01$). At the same target body weight (~2.1 kg; d34 for CONV, d48 for SLOW), CONV birds were less inactive than S-SLOW ($p < 0.01$), M-SLOW ($p < 0.01$) and F-SLOW birds ($p < 0.01$), but the inactivity levels of the S-SLOW, M-SLOW and F-SLOW birds were similar (all $p > 0.05$). At a higher body weight (~3.5 kg; d48 for CONV, d62 for SLOW), there were no differences in inactivity between strain categories. Results suggest that strain differences in inactivity may initially be due to differences in the speed of growth, with later increases in inactivity due to both age and increasing body weight. Wearable accelerometers permit the continuous monitoring of broilers to identify activity patterns that may be proxies for welfare, such as long durations of inactivity, which may be associated with poor leg health or a reduced physical ability to perform natural behaviours.

**Key Words:** behaviour, broiler, slow grow, welfare

32 Utilizing 3-dimensional models to assess keel bone damage in laying hens throughout the lay cycle. Brittney Emmert$^{1,2}$, Prafulla Regmi$^1$, Cara Robison$^2$, Kaylee Montney$^2$, Darrin M. Karcher$^1$, Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, United States, Department of Animal Science, Michigan State University, East Lansing, Michigan, United States, Department of Animal Science, Purdue University, West Lafayette, Indiana, United States.

Currently, 20% of the 340 million laying hens in the US are cage-free, and this is projected to increase as consumer demand for cage-free eggs rises. Cage-free systems have an increased incidence of keel bone damage, which may result in decreased egg production and compromised well-being by causing pain and possibly reducing their ability to effectively navigate their environment. The objective of this study was to determine the effects of dietary supplementation of n-3 fatty acids and vitamin D on keel bone damage in hens housed in a multi-tier aviary system (AV). A total of 2304 brown hens were placed in AV system after rearing at 17 wks with each room containing 4 sections of 576 birds each. At 12 wks, hens were randomly assigned to dietary treatments of FL (flaxseed oil), FS (fish oil), Vit (25-hydroxy Vitamin D3; 2760 IU/kg), and standard (AC) with one treatment/room. For each treatment, 36 focal birds were longitudinally examined for keel bone damage using computed tomography (CT) scans at nine time points starting at 16 wks until 52 wks. Mimics software (Materialise, Plymouth, MI, USA) was used to create 3-D digital twins of the keels from the CT scans. The 3-D digital twins from each time point were visually assessed for damage in MeshLab (Open Source software). For each keel, an overall severity score was recorded as well as the location, direction, type, and severity of each individual deviation or fracture. Severity was ranked on a 0-5 scale with 0 being no damage and 5 being severe. Binary and multinomial scores were analyzed using the mixed model ANOVA with main effects of treatment and age. Regardless of treatment, at 16 wks, 80% of hens had overall keel scores of 0 and 20% had scores of 1. At 52 wks, all hens had some damage, with 30% having a score of 1, 61% scored 2-3, and 9% scored 4-5. Keel deviation prevalence increased with age from 24% at 16 wks to 52% at 18 wks and 97% at 52 wks ($P < 0.01$). Fractures were not observed until 20 wks and number of hens with fractures increased at every time point after 22 wks ($P \leq 0.03$). Among the treatments, AC birds had 20% more fracture prevalence than FS birds (85% vs. 65%; $P = 0.08$), whereas Vit and FL birds were intermediate (74% and 70%, respectively). For deviations, the Vit group had more total deviation incidents compared to FL ($P = 0.05$). Overall, incorporating fish oil in the diet could reduce keel fractures, however, majority of laying hens in an AV system are still vulnerable to some form of keel damage.

**Key Words:** keel, computed tomography, laying hen, 3-D model, aviary

33 Automatic detection of reproductive behavior in male Japanese quail (Coturnix japonica) using accelerometers and neural networks. Catalina Simian$^{1,2}$, Lucas M. Barberis$^1$, Raul H. Marin*$^{1,2}$, Jackelyn M. Kembro$^{1,2}$, Universidad Nacional de Córdoba, Facultad de Ciencias Exactas, Físicas y Naturales, Instituto de Ciencia y Tecnología de los Alimentos (ICTA) and Cátedra de Química Biológica, Córdoba, Argentina, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Investigaciones Biológicas y Tecnológicas (IIByT, CONICET-UNC), Córdoba, Argentina, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Física Enrique Gaviola (IFEG, CONICET-UNC), Córdoba, Argentina.

Tri-axial accelerometers placed on an animal measure the 3-dimensional acceleration vector associated with body movements over time. When combined with machine learning and data processing techniques, such as neural networks, this methodology has the potential for classifying the recorded acceleration data into behavioral categories. Herein, we propose a system that implements the use of an accelerometer attached to male Japanese quail as a useful way for automatic detection of male reproductive behavior. Two different methods for attaching the accelerometer to
the birds were also tested. Fifteen males and thirty females were divided into one of three experimental groups: 1) control without accelerometer attached, 2) using an accelerometer attached to a backpack (i.e. harness fitted by 2 elastic fabric bands around the wings' base) or 3) using an accelerometer attached to a patch made of fabric glued to the back of the bird. All males were handled similarly and remained individually housed during a one-week period until testing. The test initiated when a male was introduced into the homebox of two female belonging to the same experimental group, during a 1-hour period. One camera above and one on the side of the box were used to record behaviors. From video-recording, a high resolution ethogram was performed defining all observable male behaviors at a 1/15s resolution during the first 10-min of testing (9000 data time points per bird). The number and duration of detected behavioral events were estimated. Accelerometer data was collected during the total 60-min of testing. General linearized models were used to assess differences between groups in the most frequently observed behavioral events, namely immobility, vigilance, shakes, exploration, walking, running, grabs, and mounts. In the vast majority of the variables evaluated no differences were observed between groups (P>0.05), including number and durations of mounts. In a second stage, the high-resolution behavioral time series registered from video-recordings were used first to train and then to validate a neural networks, to automatically detect within the accelerometer data the male reproductive events. Noteworthy, all displays of reproductive behavior during the 1-hour testing period were detected with this method. Thus, the proposed system is a first step towards automating the detection of reproductive behaviors relevant for studies where visual observations of video-recording are either not possible or impracticable. In particular, this methodology could be useful to assess male reproductive patterns over time within different social and environmental contexts.

Key Words: Japanese quail, social behavior, reproduction, accelerometry, remote detection systems

In commercial broiler houses, animal floor uniformity and distribution in drinking, feeding, and resting zones are critical information for evaluating flock production, health, and wellbeing. Proper distribution in the house is an indication of a healthy flock. Daily routine inspection of broiler flock distributions is done manually in commercial grow-out houses, which is labor intensive and time consuming. This task requires an efficient system that can monitor birds’ floor distributions automatically. In this study, a machine vision-based method, i.e., back propagation (BP) neural network algorithm, was developed and tested in a research broiler house at the University of Georgia. Six identical pens (1.84 L×1.16 W m) were used to raise Cobb-500 broiler chickens (21 birds per pen) from d1 to d49 to test the new method. Each pen was monitored with a HD camera (PRO-1080MSFB, Swann Communications Santa Fe Springs, CA) on ceiling (2.5 m above floor) to capture video (15 frame/s with the resolution of 1440 ×1080 pixels) of group birds. For machine-vision based method to recognize birds’ distribution on images, the pen floor was virtually defined/divided as drinking, feeding, and activity/rest zones. Broiler management (e.g., feeding, drinking, lighting, and house air temperature) followed the industry standards. As broiler chickens grew, images collected from each individual day are analyzed separately to avoid the bias caused by change of body weight/size over days. From a whole flock cycle test, about 7000 high quality images from d18-d35 were selected to build the BP network model for floor distribution analysis. Images from each day were randomly selected for model validation. A one-way ANOVA was used to test if there were significant differences in the detection speed of broiler chickens on the same image between the method developed in the current study, K-means, and FCM. The effect was considered to be significant when P-value was less than 0.05. Results showed that the identification accuracy of birds’ distribution in the drinking and feeding zones are 0.954 and 0.942, respectively, which provides a tool to quantify the time budget of animal feeding and drinking behaviors for improving equipment design. The current method required less time in clustering than K-means and FCM methods (p<0.001). The correlation coefficient R, mean square error (MSE), and mean absolute error (MAE) of the BP model were 0.996, 0.038, and 0.178, respectively, in analyzing broiler distribution on pen floor. The missed detection was mainly caused by blocks or interferences of facilities (e.g., feeder hanging chains and drinker pipes) on chickens’ floor images. Studies are ongoing to address those interreference issues.

Key Words: Broiler chicken, health and welfare, animal behaviors, precision farming
Extension and Instruction

35 Writing assignments in undergraduate poultry production curricula. Ruediger Hauck*1, 2, Amit Morey2, Emefa A. Monu2, Christopher Basgier1, Amy N. Wright1, 1Department of Pathobiology, Auburn University, Auburn, Alabama, United States, 2Department of Poultry Science, Auburn University, Auburn, Alabama, United States, 3Office of University Writing, Auburn University, Auburn, Alabama, United States, 4Department of Horticulture, Auburn University, Auburn, Alabama, United States.

The majority of Poultry Production students will work in supervisory and managerial positions where they will need to utilize effective written communication skills. The increasing size and evolving management systems increase the importance of appropriate expression in writing to employees at different levels and for a variety of purposes. In their professional environment, the students must be able to write different types of documents, including in-depth reports, memo-style compilations of facts and instructions like Standard Operation Procedures (SOPs). The aim of this project is to coordinate efforts and to strengthen the reports, memo-style compilations of facts and instructions with the overarching maxim that “Students who take the courses associated with this project will understand how written information is communicated to different audiences in the industry”. SLOs described conducting a literature research, preparing instructions and generating recommendations based on data collection. Writing assignments in the courses were then created or re-designed to align with the SLOs. The assignments were formulated to clearly communicate their purpose and the rhetorical situation to the students. The assignments are centered on a common topic from "farm to fork" related to food safety and quality. To make the assignments more relevant and to better visualize the interconnectedness of different aspects of the poultry production and processing systems, the assignments were designed to be documents in a virtual company. This uniquely designed company has its own web presence, which presents basic information about the company, provides material to the students and presents selected results of student assignments. This format helps the students to recognize the practical relevance and distinguish which type of document is appropriate in a given situation. The results are assessed using as metrics the performance of students in rubrics aligned with the SLOs.

In spring 2020, assessment was completed in Principles of Food Safety, the only participating class taught in spring, using 29 students. When overall scores in the Literature Review assignment were compared to that of the previous year, the average rose by 5%. Assessment of individual categories of the rubric showed the performance criteria with the most improvement was Citations, where the average score increased by 21%. In conclusion, these results show that the approach improves the students’ writing skills.

Key Words: Teaching, Writing, Standard Learning Outcomes

36 The Elementary E.G.G. Program: Impacting student interest through poultry science STEM-curriculum. Danielle Marks*, Elizabeth Karcher, Animal Sciences, Purdue University, West Lafayette, Indiana, United States.

The demand for egg and poultry protein is continuously increasing while the rising need for egg industry professionals is not being met. Relatively low interest in poultry science careers can be linked to a lack of exposure and low general knowledge of the industry. In order to increase exposure to the industry, we created a STEM-based elementary program focused on using real-world poultry science concepts to stimulate interest in the egg industry. The Elementary Education, Gain, Grow (E.G.G.) program consisted of integrating required STEM curriculum with five online modules, a supplemental interactive notebook, an embedded simulation game, and a team project. The program was piloted in the Fall of 2019 in nineteen 4th and 5th grade classrooms (480 students) across eight Indiana districts. The program was designed to be completed in ten consecutive days with students engaged approximately 45 minutes each day. Students completed the online modules and interactive notebooks from days 1 to 5 and the team project on days 6 to 10. A pre-program (Day 0) questionnaire assessed student individual interest scores while post-module (Day 5) and post-program (Day 10) assessments evaluated students’ situational interest in relation to engaging with the online modules or the team project. Student situational interest (n=111; 23.1% response rate) was elevated when engaging in both the online modules and the team project with all scores above a two out of a possible four on a validated four-point Likert scale. A paired t-test indicated that subscales assessing levels of attention, challenge, novelty, and overall scores were higher in the team project compared to online modules (p < 0.05). Qualitative data from student notebook responses (n=172; 35.83% response rate) was collected and inductively coded for student interest themes related to each module and the overall program. Student interest themes reflected modules’ learning objectives with students demonstrating repeated interest in hen anatomy and animal welfare. Overall, the Elementary E.G.G program curriculum successfully impacted student situational interest while engaging in activities within the context of the egg industry. By successfully stimulating situational interest in students when engaging with poultry sciences, there is potential to cultivate a sustained individual interest which supports possible career choices. Future longitudinal research is
needed to explore how involvement in a STEM integrated poultry science program impacts student involvement in future related career opportunities.

**Key Words:** Situational Interest, Egg Industry, STEM-Integration, Curriculum, Education

37  **Poultry Products: An on-line undergraduate course.** Glenn Birrenkott*, Richelle Miller, Animal & Veterinary Sciences, Clemson University, Clemson, South Carolina, United States.

Our undergraduate curriculum requires all students to complete a capstone course in Animal Products or Production. Our Animal Products course is 3-credits and partitioned into 3 sections: Dairy, Meats, and Poultry products. We have 2 lectures and a 2-hour lab each week for about 70 students. The 4 weeks of Poultry Products consists of: 1 What is the Poultry Industry, 2 Reproductive Products (eggs and turkey testicles), 3 Muscle (skeletal, giblets) and 4 Integument (feathers & leathers). The COVID pandemic shut down our on-campus lectures and labs at the transition from Meats to the Poultry section. We used this opportunity to explore different delivery methods for poultry lecture and lab material while using the Course Analytics that we have used to assess student engagement and grades for the previous 3 years of on-campus delivery. We created 20 to 30-minute voice-over presentations and uploaded them to YouTube as Unlisted. Our students could access these lectures only when we provided them the URLs. We supplemented with Zoom recordings of poultry lecture and lab materials. To encourage our students to stay engaged and keep up with our lesson plans we created specific 10-point graded assignments based on their participation in the weekly discussion topic. A typical assignment was their submission of a specific number of T/F, multiple choice and fill-in questions (with correct answers) on the Poultry topic of the week. Only after they submitted their contribution could they see all participants’ questions, answers and comments. This created a weekly cache of 300+ potential exam questions. The on-line Poultry Products final exam was given on-line in 4 modules corresponding to the syllabus topics. A measure of student engagement is the number of page views during the semester. In 2019, we had a maximum of 2200-page views during one week. This semester we exceeded 3800-page views each week during the Poultry Products section. We had a 95% participation rate in our Poultry weekly discussion topics. These were factors in the higher test scores in the Poultry section and a higher overall distribution of A and B grades this semester when compared to data from the previous 3 years. Student feedback was positive. They liked the interactions with instructors and peers and being able access lecture and lab materials at their convenience. But they were rightly critical of the pace of initiating on-line lectures which compressed some of the topics at the end of the semester. Students were also disappointed in not having hands-on lab opportunities – because they pay lab fees for our courses and they missed out on the cooking and consumption of some of the poultry products!

**Key Words:** Integument, Poultry Products, On-line course

38  **Transitioning an upper level poultry management course from in-person to online instruction during the COVID-19 outbreak.** Jacqueline Jacob*, Anthony Pescatore, Sunday Adedokun, Animal and Food Sciences, University of Kentucky, Lexington, Kentucky, United States.

The objective of this report is to discuss our experience in transitioning a live poultry management course to online in a short period of time. The University of Kentucky offers a 2 credit upper level poultry management course that is a weekly 90-minute lecture. This course fulfills the requirement for an animal production course in the Animal Science (ASC) degree. Enrollment was 42 students with 90% female and 80% seniors. The students were 78% ASC majors, 17% ASC minors. Attendance was monitored and rewarded with bonus points. Students were expected to complete three assignments, two exams and a comprehensive final. The course utilized a Canvas Dashboard to post assignments, lecture notes and grades. At the midpoint of the semester, the university announced that the students would not be returning to campus. Courses were expected to transition all instruction to online in ten days. The short transition time, coupled with the additional stress on the students required the course to be flexibility. Live Zoom lectures were eliminated because of concerns about student internet access, students in numerous time zones and minimal access to technical support. The existing Canvas Dashboard was reconfigured, and the remaining scheduled lectures were posted as narrated PowerPoints. The narrated PowerPoints allowed both the professors and students flexibility in scheduling their online work. The assignments in the course have always been designed to serve as study guides, with the transition to online, assignments were changed from a crossword puzzle to practice exams that the students could take multiple times. Regular exams were held online, during normal class time, with a set time limit. Students with approved requests for accommodations had adjusted test time. Antidotal experience with the two assignments indicated no change in the assignment scores. The average number of times the practice exam was attempted was 2.9±1.3. For exam 1, which was taken in person and proctored, the average score was 121.1±12.0/150 with a range of 94.5 to 148.5 and a median of 122.3 points. The online exam, which could only be taken once, and during what would have been the class time, the students averaged 135.0±11.0/150 with a range of 103.5 to 148.5 and a median of 138.8 points. There was a numeric improvement of 9.2±8.0 percentage points in test scores between exam 1 and 2 but changes ranged from +24 to -8. The majority, 34 students, had increases in test scores, seven had reduced scores. Although we utilized a random selection from a pool of questions and a tight exam time frame, we cannot rule out that the improvement in tests
scores was due to access to lecture notes which we did not actively discourage.

**Key Words:** Handing change, online, poultry management course

### 39 Measuring interest and engagement in Extension programming.

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In extension programming, it is crucial to use evidence-based methods to optimize participant learning. However, there is limited implementable strategies to review and quantify programming impact on participants. The objective of our study was to evaluate and describe participant interest and engagement in the inaugural offering of the Shell Egg Academy. We randomly selected eight sessions (25-40 participants/session) across the 16 total sessions offered and evaluated participant engagement, interest, and motivation. Instructor (n = 8) teaching type was recorded during each of the sessions and each instructor taught only one session. A paper questionnaire was administered immediately following each session and contained 47 questions. Likert-style questions to evaluate interest, motivation, and self-reported engagement. Sessions were recorded and reviewed using the Behavioral Engagement Related to Instruction protocol in order to observe actual participant engagement. Overall, instructors selected lecture as the primary instructional method, providing little variation in teaching strategies. Participant situational interest, including subfactors of exploration intention, instant enjoyment, and attention demand, was moderate to high during all sessions. Intrinsic motivation and identified regulation were also moderate to high during the sessions, indicating that participants felt a personal importance and natural enjoyment during sessions. Participant observed and self-reported engagement did not differ between sessions. This study is one of the first to describe participant interest and motivation when engaging in an extension program. Additional work is needed to better understand the impact of adopting different instructional strategies on participant engagement, interest, and motivation in order to maximize participant experience and build evidence-based program evaluation practices.

**Key Words:** Interest, Motivation, Engagement

### 40 Promotion of small-scale poultry farming through university extension in northern Rio de Janeiro, Brazil.

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The project was a joint effort between the public sector (Poultry extension of Norte Fluminense State University and the Agriculture Secretariat of Campos, Rio de Janeiro state) and rural landholders. The aim was to promote regional small-scale poultry farming. Two phases were development: The first (2014 to 2017) involved incentives to poultry production aimed at members of an agrarian reform movement, through the grant by free loan for use (commodatum) of 10 small open plots, equipped for rearing broilers, birds, and inputs for two cycles, under technical monitoring. Theoretical-practical training and a socioeconomic questionnaire were administered first. The participants (110) began the process, but many dropped out during the training, mainly due to mistaken interpretation of free delivery of birds and inputs as the main objective of the project. Only 16 continued to the socioeconomic classification phase. The participants stated that the main motive to participate was the opportunity for work, but unwillingness to pay the costs. This last response indicated possible underestimation of “having access to public policies/social programs.” The visit to the property helped in the classification. In one case we observed that the small farming operation had more basic resources than the participant’s dwelling, so the extension work helped to identify basic problems of human development. Nine small operations were established, but only three people managed to finalize all the phases. Hereafter 2017, the second phase, there was no granting of incentives and the flow of the project changed: enrollment (20), free training (17), a technical visit to the property (12), planning (12), and implementation of the project (9), orientation for the legalization of the activity (7), and follow-up monitoring (7). The project provided installations, equipment, and labor to manufacture feed. The operations were smaller and were adjusted to the characteristics of the property and investment capacity. The schooling level was higher, since some of the participants worked as technicians for oil companies, with solvency and participation in the planning and development of the project, irrespective of the expectation to receive public resources. As a negative point, there was little interest in the formation of groups or associations to work as small poultry farmers. The two experiences of the extension work in poultry farming showed that the way the participants were approached led to different objectives. In the first case, there was a strong expectation from the grant itself of a previously planned project, while in the second the participants were active in the planning and managed to continue until the follow-up phase.

**Key Words:** Public policy, Regional development, Technology transfer

### 41 Evaluating the causes of arthritis condemnation in a Brazilian slaughterhouse.

Sanitary and other management problems can partially or totally compromise the utilization of a broiler chickens’ carcass in a slaughterhouse. In this context, an effective inspection of the produced meat is important, ensuring the product reaches the consumer with quality assurance and pathogens free. Collecting, measuring, and knowing the main carcass compromising causes is fundamental to improve methodologies and practices to avoid and attain better results in relation to that issue. An important cause of total or partial carcass condemnation is arthritis, which may have multiple origins and deserves special consideration.

The objective of this field study is to evaluate the occurrence of arthritis and evaluate its relationship with livestock density, slaughter weight, slaughter age, and gender inside an abattoir in the south of Brazil. The abattoir where the data was collected slaughters around 72,000 broiler chickens per day. This study analyzed a total of 4,680,000 broiler arthritic condemnation values from August to October 2019. Data were collected daily from the staff at the inspection line. The SAS 9.4 (2009) software was used for the statistical analysis using the proc GLM and proc Univariate to access the relationship between arthritis and collected variables. Results show the average partial condemnation for arthritis was 3.17%. Lower livestock density resulted in significant lower manifestation of arthritis problems when compared with higher densities (3.6 and 4.85% respectively). Heavier and older birds were more attained by articular problems than lighter birds (3.01 and 5.0% for 2.6kg and 3kg respectively). Birds gender do not show any statistical difference. In this study we can conclude that lower arthritis incidence can be obtained with lower bird weights and livestock densities.

Key Words: carcass condemnation, arthritis, broiler chicken

42 Possibilities for formulating layer diets beyond the traditional least-cost model. Amy F. Moss*,1, Greg Parkinson*,1, T. M. Crowley2,3, Gene M. Pesti1,1University of New England, Armidale, New South Wales, Australia, 2Poultry Hub Australia, University of New England, Armidale, New South Wales, Australia, 3School of Medicine, Deakin University, Geelong, Victoria, Australia, 4Livorno Consulting, Brunswick, Victoria, Australia.

Feed constitutes >65% of live production costs in poultry production; thus there is vast pressure to formulate ‘least-cost’ diets. The aim of commercial enterprises is usually to maximise profits with the inputs available. In this respect, least-cost feed formulation is limited as it does not account for the relationship between input cost and output value. While it was previously necessary due to the complexity of non-linear models, modern computing has made max-profit feed formulation achievable. Importantly, these models improve flexibility and economic sustainability which is particularly of interest during the recent market volatility.

Therefore, this study compared diets formulated in traditional least-cost versus max-profit approaches via practical simulations. The layer hen simulation focuses on dietary methionine (Met) levels as it is an expensive dietary constituent and holds important implications for egg size. The response of intake, egg weight, total egg mass and percentage production of cage layers 52-58 weeks of age to 5 true digestible Met levels (0.13, 0.25, 0.37, 0.48, 0.6%) was sourced from Bregendahl et al. (2008) to model the response of layers over the 6 week period. Diets reported were reconstructed using modern prices (4th quarter 2019, SAUD) sourced from industry. The standard least-cost diet formulated to standard nutrient recommendations consisted of 0.48% Met costing $461.26, resulting in an estimated egg mass of 52 g/bird/day and an egg production of 83%. Therefore, a farm of 20,000 hens from 52-58 weeks of age (42 days) may expect to produce a total of 58,100 dozen eggs (on average 700g egg grade) with a sale price of $2.20/doz. Variable and fixed costs were calculated at $93,575 and revenue (egg sales + spend hens) at $128,220 giving a profit of $34,645 for the 6 week least-cost simulation. Calculations were repeated for all Met levels reported in Bregendahl et al. (2008) and the highest dietary Met level of 0.6% generated the greatest profit of $34,830. However, the maximal profit achievable is defined as the point at which more Met inclusion won’t generate any extra profit, or the point at which marginal costs equal marginal revenue. As the reference data did not cover this range, the response of layers to Met was modelled and the point at which marginal costs equal marginal revenues was estimated at 0.95% dietary Met. Thus, the most economic nutrient level may not correspond with the biological nutrient recommendation, and the greater flexibility and revenue provided by max-profit formulations may aid the poultry industry particularly during challenging economic periods.

Key Words: Diet, Formulation, Least-cost, Max-profit, Layer

43 Smallholder farmer community-based breeding program of indigenous chickens key to in-situ genetic resource conservation and enhancing rural livelihoods in Zambia. Christopher M. Kanyama1*,2, Amy F. Moss1, TM Crowley2,1University of New England, Armidale, New South Wales, Australia, 2Poultry Hub Australia, University of New England, Armidale, New South Wales, Australia. Agriculture is the primary livelihood for many nations. Most rural communities in Sub-Saharan Africa (SSA) depend on agriculture for their livelihood but are also custodians of essential animal genetic resources in the region. Nearly 80% of smallholder farmers rear small livestock, including indigenous chickens (IC) (Gallus domesticus) for household income, food and nutritional security. However, populations of IC genetic resources over the past decade have experienced erosion and extinction and thus have the potential to affect future production and biodiversity negatively. Among the avian species, many
chicken varieties are in danger with 62% of chicken strains being of unknown status, 33% at risk of extinction and 3.5% extinct. These negative changes can adversely affect rural communities in SSA. Therefore, genetic resource conservation focused on in-situ selection, development, multiplication and utilisation of Indigenous chickens in rural communities should be considered. In SSA, Zambia has also experienced poor performance, including loss of breeds of IC attributed to disease and weak markets dominated by intensive broiler and egg chicken production. Therefore, this research project will focus on a community-based breeding program in Zambia to formulate solutions for local smallholder farmers to mitigate the losses of IC genetic resources. The three main objectives are to; i) strengthen sustainable selection, multiplication, utilisation and conservation of IC, (ii), improve efficiency in production and disease control of IC (iii), and explore potential market value chains of IC. The methodology will involve undertaking a survey, focus group discussion, and interviews for 50 households to collect social-economic data throughout 2021. This data will include breeds reared, production systems used, common poultry diseases, and marketing systems practised. Identification of challenges faced by farmers involved in IC production will be through the engagements and consensus of the research team. A bio-economic model will then be used to evaluate the profitability and economic contributions of the essential biological traits concerning consumer-market demands (Okeno et al., 2012). The model will describe the production system used and the effects of biological characteristics on costs due to labour, veterinary, brooding, feeding requirements and revenue from the sale of eggs, growers, and culled breeding stock. This research aims to facilitate future benefits for both smallholder farmers through improved livelihoods, and consumers are having access to healthy IC eggs and meat from the market.

**Key Words:** Genetic resource, Indigenous chickens, Conservation, Sustainability, Rural community
Comprehensive analysis of circular RNAs related to hypoxic adaptation in the Tibetan chicken. Ying Zhang, Woo Kim, Woyu Su, Hao Zhang, China Agricultural University, Beijing, China. University of Georgia, Athens, Georgia, United States.

Tibetan chicken has a suite of adaptive features to tolerate the high-altitude hypoxic environment as a unique native breed in Qinghai-Tibet Plateau of China. The Chahua chicken is domesticated from the red jungle fowl, which is a low-altitude typical local chicken breed in Yunnan Province. The Chorioallantoic membrane (CAM) that functions as a respiratory and circulatory organ of chicken embryo has critical roles for embryonic survival and development during incubation. Circular RNAs (circRNAs) that are important members of the non-coding RNA family involve in regulation of various physiological functions. To investigate the role of circRNAs in hypoxic adaptation, RNA sequencing was performed in CAM of Tibetan (TC) and Chahua chicken (CH) embryos that were both incubated in hypoxic (13% O2) condition. A total of 1414 circRNAs that are important members of the non-coding RNA family were identified and in which more than 70% of circRNAs were derived from exon. Comparing between the TC and CH, 93 differentially expressed circRNAs (DECs) were obtained with |log2FC| > 1 and P < 0.05. Based on target prediction and functional enrichment, some DECs, such as 3:109878386/109891500, 10:17468696/17474782, 6:33581680/33588236, and 21:6554567/6555014, might play important roles in the hypoxic adaptation. Several host genes of DECs are involved in blood vessel development, angiogenesis, immune response, glucose metabolic process, carbohydrate metabolic process, and MAPK and notch signaling pathways. Six host genes (ADGRF5, MEF2A, ADAM12, HSPG2, AOPEP, and CEMIP2) function in promoting angiogenesis and inducing vascular morphogenesis. Six host genes (ADGRF5, NLN, TMEM2, GXYLT1, ARFGEF1, and ISPD) participate in glucose and carbohydrate metabolic processes. These results indicated that the embryos of Tibetan chickens might regulate angiogenesis and energy metabolism through several key circRNAs and their host genes to adapt the hypoxic condition. Our results provide new insights into the molecular mechanisms for hypoxic adaptation in chicken embryos and understanding of hypoxic diseases in humans, and the identified circRNAs and target genes needed to be further investigated for functional mechanisms.

Key Words: circRNA, Tibetan chicken, hypoxic adaptation, angiogenesis, host genes


This study was conducted to explore the relationship among body measurements in 4 strains of broiler chicken (Arbor Acre, Hubbard, Marshall and Ross 308) using principal component analysis with the view of identifying those components that define body conformation in broilers, using stepwise discriminant analysis and cluster analyses to assess the magnitude of genetic diversity and heritability.
Winter adipose development in broiler chicks. Adipose samples from the abdominal, subcutaneous incubated to embryonic days 13, 15 and 17 (E13, E15, E17, respectively) or hatched and grown to 7 or 14 d of age (n=6-8/age). Adipose samples from the abdominal, subcutaneous (SQ), and neck depots and liver samples were snap-frozen for RNA isolation and cDNA synthesis. Expression levels of chicken SIRT genes 2, 4, 5, 6, and 7 (SIRT2, 4, 5, 6, and 7) were quantitated using targeted RNA sequencing, as part of a larger gene panel. Raw sequence reads were aligned to the reference genome (Gal_gal-5.0), counted, and normalized to the geometric mean of three housekeeping genes (ACTB, YHAP, and TBP). Effects of embryonic through post-hatch development on gene expression in SQ adipose tissue were analyzed using single factor ANOVA (SAS 9.4). Differences between adipose depots and liver at 7 and 14d were analyzed using two-factor ANOVA (tissue and age) with interaction. Least squares means (LSM) were used to detect significant pairwise differences between groups (p ≤ 0.05). Correlations between genes were calculated using Spearman’s rank correlation. Expression of each of the five SIRT genes varied significantly with age in SQ adipose and showed unique profiles of expression from E13 to D14. Marked changes in expression for SIRT2, 5 and 6 were detected at D7 relative to E17 and to D1. After hatch, expression of each SIRT gene was significantly affected by interaction between age and tissue type, particularly at D7, a period of dynamic metabolic and diet adaptation. Expression levels in adipose after hatch were comparable to those in liver. SIRT4 and SIRT7 were highly correlated with genes related to adipogenesis and fatty acid uptake, and inversely related to genes (e.g., CPT1A, ACOX1) that mediate fatty acid oxidation. Conversely, SIRT2, 5 and 6 were inversely related to genes that mediate fat accretion. In conclusion, these results demonstrate that the SIRT family of enzymes is dynamically regulated during adipose development in broiler chicks. Given their established roles in mammals, this class of enzymes may represent an additional target through which to reduce fat accretion and enhance metabolism in broilers.

Key Words: siruin, adipose

47 Siruin genes are dynamically regulated during adipose development in broiler chicks. Heather Wintert, Robert Mihelic, Suchita Das, Kurt Lamour, Brynn H. Voy, Animal Science, University of Tennessee, Knoxville, Tennessee, United States, Entomology and Plant Pathology, University of Tennessee, Knoxville, Tennessee, United States.

Siruins are a family of highly conserved protein deacetylases that couple metabolic status to histone modifications through their absolute requirement for nicotinamide adenine dinucleotide (NAD+). In other species, siruins are closely tied to regulation of adipogenesis, but they have not been well-characterized in chicken. Our objective was to define the relative expression patterns of chicken siruin (SIRT) genes during embryonic and post-hatch adipose development as a step toward understanding their role in fat accretion and metabolism in broilers. Fertilized commercial broiler eggs were either incubated to embryonic days 13, 15 and 17 (E13, E15, E17, respectively) or hatched and grown to 7 or 14 d of age (n=6-8/age). Adipose samples from the abdominal, subcutaneous (SQ), and neck depots and liver samples were snap-frozen for RNA isolation and cDNA synthesis. Expression levels of chicken SIRT genes 2, 4, 5, 6, and 7 (SIRT2, 4, 5, 6, and 7) were quantitated using targeted RNA sequencing, as part of a larger gene panel. Raw sequence reads were aligned to the reference genome (Gal_gal-5.0), counted, and normalized to the geometric mean of three housekeeping genes (ACTB, YHAP, and TBP). Effects of embryonic through post-hatch development on gene expression in SQ adipose tissue were analyzed using single factor ANOVA (SAS 9.4). Differences between adipose depots and liver at 7 and 14d were analyzed using two-factor ANOVA (tissue and age) with interaction. Least squares means (LSM) were used to detect significant pairwise differences between groups (p ≤ 0.05). Correlations between genes were calculated using Spearman’s rank correlation. Expression of each of the five SIRT genes varied significantly with age in SQ adipose and showed unique profiles of expression from E13 to D14. Marked changes in expression for SIRT2, 5 and 6 were detected at D7 relative to E17 and to D1. After hatch, expression of each SIRT gene was significantly affected by interaction between age and tissue type, particularly at D7, a period of dynamic metabolic and diet adaptation. Expression levels in adipose after hatch were comparable to those in liver. SIRT4 and SIRT7 were highly correlated with genes related to adipogenesis and fatty acid uptake, and inversely related to genes (e.g., CPT1A, ACOX1) that mediate fatty acid oxidation. Conversely, SIRT2, 5 and 6 were inversely related to genes that mediate fat accretion. In conclusion, these results demonstrate that the SIRT family of enzymes is dynamically regulated during adipose development in broiler chicks. Given their established roles in mammals, this class of enzymes may represent an additional target through which to reduce fat accretion and enhance metabolism in broilers.

Key Words: strain, morphometric parameters, loadings, variance maximizing orthogonality, cluster

48 Developmental expression of genes within the insulin-like growth factor system suggests they play unique roles in regulating breast muscle myogenesis and growth. Lauren A. Vaccaro, Emma England, Abigail M. Wilson, Laura Ellestad, Poultry Science, University of Georgia, Athens, Georgia, United States.

The somatotropic axis is essential in regulating the growth and metabolism of birds and mammals. Hormonal signaling of this axis is carried out in part by insulin-like growth factor (IGF) 1 and 2 activation of their receptor, and this is regulated by IGF binding proteins (IGFBPs). Previous work evaluating somatotropic axis gene expression in modern and heritage broilers suggested that genetic selection has altered expression of several genes in breast muscle, indicating that they likely play an important role in regulating development and physiology of this tissue in commercial broilers. This study’s objective was to examine developmental expression patterns of IGF1 and IGF2, IGF receptor (IGF1R), and IGFBPs in commercial broilers throughout the latter half of embryogenesis and early post-hatch. Breast muscle (Pectoralis major) was collected from 12 birds on
embryonic day (e) 12, e14, and e16 and six males on e18, e20, and post-hatch days (d) 0, d1, d3, d5, d7, d10, d14, and d21. Embryos from e12–e16 were molecularly sexed using PCR. Total RNA was extracted from 6 males at each age (n=6). Expression of mRNA for each gene was determined by reverse-transcription real-time quantitative PCR, and their levels were normalized to 18S rRNA. 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≤0.05).

Age-induced differences in expression were observed for IGF1, IGF1R, IGFBP1, IGFBP2, and IGFBP4. Expression of IGF1 was greatest at e12 and d7, while it was lowest at d0 (P≤0.05). Expression of IGF1R, IGFBP1, and IGFBP2 was raised during embryonic development but gradually decreased and was diminished after hatch (P≤0.05). Similarly, IGFBP4 remained stable through d1 and decreased thereafter (P≤0.05). During embryonic myogenesis, progenitor cells differentiate into myoblasts that fuse into multi-nucleated muscle fibers as the muscle tissue increases in size. Although fiber size can change after hatch, no new fibers are formed. The IGFs are known inducers of muscle proliferation and growth. In this context, greater IGF1 expression post-hatch is likely regulating muscle fiber growth in a paracrine manner. Heightened embryonic expression of the IGFBPs suggest they function in a paracrine manner to regulate embryonic myoblast differentiation, proliferation, and muscle fiber maturation. The decrease in IGFBP expression after hatch may serve to enhance muscle tissue sensitivity to circulating or local IGF1, despite reduced levels of IGF1R. In conclusion, the distinct expression patterns of these genes suggest they play unique roles during growth and development of muscle tissue.

**Key Words:** myogenesis, growth, development, paracrine signaling, hormones

**49 RNA sequencing identifies differential gene expression in broiler breeders managed on different feeding programs.** Chandler Keck, Martin J. Zuidhof, Nicole Zukiwsky, Mohammad Afrouziyeh, Chris Ashwell, North Carolina State University, Raleigh, North Carolina, United States, University of Alberta, Edmonton, Alberta, Canada.

Poultry breeders have successfully selected for increased efficiency and rapid growth of broilers. This success has required broiler breeder management to include restricted feeding during rearing to maintain reproductive capacity. Though this practice has been the industry standard, it is thought to cause metabolic stress and issues with welfare in regard to hunger and feeding distress. This study focuses on the impact of precision feeding on hepatic gene expression in broiler breeders. Ross 708 breeders were reared using three different feeding programs: Control- fed to reach Ross 708 breeder target growth curve; +20%- fed to reach 20% higher body weights over Control; and Ad lib – fed ad libitum. Birds from all treatments were individually fed using a precision feeding system. Liver samples were collected from three breeder hens at 44 wk of age from each of the treatment groups. RNA was isolated from the liver samples and RNA sequencing was performed resulting in an average of 13.6 million reads per sample. CLC Genomics Workbench was utilized for mapping, annotation and statistical comparison of the RNAseq data for all pairwise comparisons. Ingenuity Pathway Analysis was used to further characterize the CLC results. Using a minimum absolute fold change of 1.5 and p-value of 0.05 as a threshold for significant differences, 600 differentially expressed genes were identified in the Control vs. +20% comparison; 1,524 in the Control vs. Ad lib comparison; and 1,217 in the Ad lib vs. +20% comparison. Pathway analysis suggests that the primary system impacted by treatment was the regulation of reproductive hormones. Other systems that were altered across all pairwise comparisons included lipid metabolism and stress response pathways. Potential common upstream regulators of these systems that had a significant p-value of overlap of differentially expressed downstream molecules in one or more pairwise comparisons included betaestradiol, progesterone, luteinizing hormone, follicle stimulating hormone, ghrelin, leptin, and insulin. These upstream regulators, impacted by feeding program, suggest that breeders may be using multiple approaches to adapt to food availability, with the regulation of gene expression downstream of reproductive hormones being the most significant. Our findings also indicate that the +20% treatment is more similar to the control group than the Ad lib group. These findings in broiler breeders warrant further research into the mechanisms of action of current management practice and provide a novel insight into the biological basis of adaptation to restricted feeding programs.

**Key Words:** RNAseq, Broiler Breeder, Gene Expression, feeding program, Restricted feeding

**50 Effects of maternal immunization against myostatin on the post-hatch growth performance of their chicks.** Rajeev K. Mishra, Rajesh Jha, Birendra Mishra, Yong-Soo Kim, Human Nutrition, Food and Animal Sciences, University of Hawaii at Manoa, Honolulu, Hawaii, United States.

Myostatin (MSTN) is a negative regulator of skeletal muscle growth. A previous study showed that in ovo administration of monoclonal anti-MSTN antibody increased post-hatch body weight (BW) and muscle mass, demonstrating the potential of in ovo MSTN suppression to improve the growth performance of chicks. Therefore, we hypothesized that active immunization of hens against MSTN will produce eggs containing anti-MSTN antibodies, leading to the improvement of skeletal muscle growth of their chicks. Chicken MSTN (chMSTN) cDNA was synthesized for E. coli expression and ligated into the pET-45b(+) plasmid, and transformed into an E. coli for expression. Recombinant chMSTN was processed for hen immunization. Nine 5-month-old Hy-line Brown hens were...
divided into three groups (Control, MSTN, and Myo2) and housed with one 5-month-old White Leghorn rooster in each group. Hens were immunized by subcutaneous injection (200 µL) of the following immunogens emulsified with adjuvant on day 0 and 7: 0 and 0.5 mg of chMSTN for the Control and MSTN groups, and 200 mg of KLH-conjugated Myo2 peptide composed of amino acid sequence 50-64 of MSTN for the Myo2 group. Antibody titer against chMSTN in serum and egg yolk was monitored using ELISA. The peak antibody titer was seen on day 21 from the first immunization and the peak egg yolk antibody titer was on day 28. Thirty three eggs were collected from each group between day 24 to 34 after the first immunization and were incubated under standard conditions. Chicks were reared under a deep litter system for 11 weeks with weekly BW monitoring. At necropsy, viscera were removed, breast muscle and bone-in legs were separated and weighed. The data were analyzed by one-way ANOVA using the Graphpad PRISM program. Statistical significance was set at P<0.05. The BW of the MSTN (1312±18.7 g) group was statistically not different from that of the Control (1351±22 g), but the BW of the Myo2 (1237±18.3 g) was significantly lower than that of the Control. Similarly, carcass, breast muscle, and bone-in leg weights of the MSTN were not different from those of the Control, but those of the Myo2 were significantly smaller than those of the Control. In Western blot analysis, serum and egg yolk IgY showed binding affinity to the recombinant chMSTN but no affinity to a bioactive MSTN, suggesting that the antibodies from the MSTN and Myo2 groups have little affinity to bioactive MSTN. The lack of affinity to bioactive MSTN of the serum and IgY from the MSTN group appears to explain the lack of affinity to bioactive MSTN and Myo2 groups have little affinity to bioactive MSTN.

Key Words: myostatin, Immunization, Hen, Anti-myostatin antibody, Growth performance

51 Heat stress dysregulates intestinal mitochondrial function and dynamics in broilers. Reagan N. Cauble***, Elizabeth Greene, Sami Dridi, Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States.

High environmental temperature is one of the most challenging stressors to the poultry industry. The adverse effects of heat stress on broiler productivity, meat yield and quality, well-being, and mortality is well documented. Emerging evidence indicates that heat stress may also induce leaky gut syndrome, however the underlying mechanisms are not well defined. The present study aimed to determine the effects of heat stress on gut integrity and to define the fundamental signaling pathways. One-day old Cobb 500 broiler chicks were allocated randomly into 12 environmental chambers (2 pens/chamber and 20 birds/pen) and chambers were assigned to either heat stress conditions, or control. Chicks were reared under standard conditions. The rearing temperature was gradually decreased from 90°F for days 1 to 3, 87°F for days 4 to 6, 84°F for days 7 to 10, 78°F for days 11 to 14, and 75°F thereafter. On day 28, the ambient temperature was increased within 10 minutes to 95°F in half of chambers to induce heat stress, with the remaining chambers maintained at 75°F. Two hours after the onset of heat stress, birds were euthanized by cervical dislocation and intestinal segments were collected, snap frozen in liquid nitrogen and stored at -80°C for molecular analyses. The porcine jejunal (IPEC-J2) cells were cultured and exposed to heat stress (45°C) for 2 hours. Control cells were maintained at 37°C. Leaky gut syndrome was assessed by FITC-d method. The expression of target genes and proteins were measured by real-time qPCR and immunoblot, respectively. The tight junction integrity and structure were assessed by TEER and immunofluorescence staining[RC1]. Heat stress significantly increased core body temperature and circulating FITC-d levels in broilers and reduced TEER and increased FITC-d levels in the basolateral chamber in IPEC-J2 cells, indicating a leaky gut. Heat stress upregulated the expression of HSP60/70/90 in both in vivo and in vitro studies. Biochemical analysis and immunofluorescence staining showed that heat stress altered tight junction protein expression and structure confirming leaky gut syndrome. Electron microscopy showed elongated mitochondria under heat stress condition which suggest an enhanced fusion and absence of fission. These data have been confirmed by miotofusin 1 (MFN1) gene upregulation and alterations to mitochondrial bioenergetics. Together, these results are the first evidence suggesting that heat load induces intestinal stress and alters both mitochondrial dynamics and gut integrity resulting in leaky gut syndrome.

Key Words: Gut, Heat stress, Mitochondrial bioenergetics


The peri-hatching chick undergoes a rapid and dramatic metabolic transition from embryonic to post-hatch life. The chick embryo obtains most of its energy from oxidation of yolk lipids, whereas the hatched chick most obtain most of its energy from carbohydrate rich/lipid poor feed. We recently found a number of hepatically-expressed microRNAs (miRNAs) help facilitate this shift in metabolic processes in the chick liver. While adipose tissue was initially thought to mainly serve as a lipid storage site, it is now known to carry many metabolic, endocrine, and immunological functions. Therefore it would be expected that adipose tissue is also an important factor in the metabolic switch. To that end, we used next generation sequencing (RNA-seq/small RNA-seq) and Real-Time quantitative PCR (RT-qPCR) to generate miRNome and transcriptome signatures of the adipose tissue during the transition from late embryonic to early post-hatch development. As adipose tissue is well known to produce...
inflammatory and other immune factors, we used SPF white leghorns to generate the initial miRNome and transcriptome signatures to minimize complications from external factors (e.g. pathogenic infections) and ensure the identification of bona fide switch-associated miRNAs and transcripts. We confirmed selected expression signatures of miRNA:target pairings in the adipose tissue of broilers (Ross 708) using RT-qPCR (n=6; ≥2-fold; p≤0.05; ANOVA). Using E18 embryos as a representative of pre-switching metabolism and D3 chicks as a representative of post-switching metabolism, we identified 22 miRNAs and 467 genes which were differentially expressed (n=4; ≥2-fold; p≤0.05; CLC-exact test (Robinson and Smith)) during this transition. Pathway mapping of miRNA:target pairings with reciprocal expression, suggests that a network of miRNAs work concordantly to regulate a diverse but interconnected group of developmental, immune and metabolic processes in the adipose tissue during the metabolic switch. Network mapping suggests that during the first days post-hatch, despite the consumption of feed, the chick is still heavily reliant upon adipose tissue lipid stores for energy production, and are not yet efficiently using their new energy source for de novo lipid storage. A number of core master regulatory pathways including, circadian rhythm transcriptional regulation and GH signaling, likely work together with miRNAs that regulate them, to maintain an essential balance between adipogenic, lipolytic, developmental, and immunological processes in the adipose tissue during the metabolic switch.

Key Words: development, adipose tissue, microRNA, RNA-seq

53 Genetic relationship between perching and frequency of floor eggs in brown layers. Anna Wole*1,2, Petek Settar2, Janet Fulton2, Jesus Arango2, Kaylee Rowland2, Danny Lubritz2, Jack Dekkers1. Animal Science, Iowa State University, Ames, Iowa, United States, Research and Development, Hy-Line International, Dallas Center, Iowa, United States.

As cage-free production systems become increasingly popular, behavioral traits such as nesting behavior and calmness have become more important. The objective of this study was to estimate heritability and genetic correlation between frequency of perching and proportion of floor eggs in two Rode Island Red lines. Testing was done in floor pens housed by sire family with two replicates per sire. Details about the facility are found in Settar et al., 2006. Hens perching and floor eggs were counted daily during 11 weeks of test, and the data was accumulated into weekly records to improve normality. In total, 6054 and 6442 weekly records were available across four tests for the two lines. A bivariate animal model with fixed effects of test and linear and quadratic covariate for the week of test was implemented in ASReml (Gilmour et al., 2014). Percent of the hens observed perching tended to increase and proportion of eggs laid on the floor tended to decrease as the tests progressed. This suggests the ability of hens to learn to utilize nests and perches. The estimates of heritability in the two lines were 0.18 and 0.21 for the percent of hens perching; 0.47 and 0.48 for the percent of floor eggs. Estimates of genetic correlations between perching and floor eggs were -0.10 and -0.14 for the two lines suggesting that genetically there is a tendency for hens that better utilize perches to also utilize nests. In conclusion, perching and tendency to lay floor eggs were shown to be a learned behavior stressing the importance of proper management and training of pullets and young hens. A significant genetic component was found confirming possibility of improving nesting behavior for cage-free systems through genetic selection.

Key Words: laying hens, perching, floor eggs, heritability, genetic correlation
Immunology, Health and Disease

54 Impacts of antibiotic reduction strategies on zootechnical performances, health control and *Eimeria* spp. oocysts excretion compared to conventional antibiotic programs in commercial broiler chicken flocks. Eric Parent*1,2, Marie Archambault1,2, Robert J. Moore3, Martine Boulianne1,2, "Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, Quebec, Canada, "Centre de Recherche en Infectiologie Porcine et Avicole (CRIPA-FRQNT), Université de Montréal, St-Hyacinthe, Quebec, Canada, "School of Science, RMIT University, Bundoolu, Victoria, Australia.

The objective of this prospective study was to evaluate, in commercial broiler chicken farms, two antibiotic reduction strategies eliminating the use of medically important antibiotics in comparison to the conventional use of antibiotics. On 7 commercial broiler chicken farms, one house was allocated to antibiotic reduction treatments for 6 consecutive flocks, while a similar house on each premise was assigned to the conventional use of antibiotics (CONV) for 6 consecutive flocks. Antibiotic reduction strategies consisted of continuous in-feed use of ionophores (TX1) or continuous in-feed use of ionophores with butyric acid (TX2). In all flocks (N = 84), zootechnical performance was recorded, lesion scoring using Elanco’s Intestinal Integrity (I2) index scoring system at 21 and 28 days of age was performed, and fecal samples were collected for sequential *Eimeria* spp. oocysts counts. Mixed linear regression models with the farms as random effects were built to evaluate zootechnical parameters and average lesion scores. For oocysts excretion modeling, two mixed models were built to model excretion at early ages (7, 10, 13, 16 and 19 days of age) and later ages (22, 25, 28, 31 and 34 days of age). There were no statistical differences between TX1, TX2 and CONV for final body weights, feed conversion ratios, average daily gains, age at slaughter, total mortalities and condemnations (p > 0.05). The probability of identifying oocysts in fecal samples significantly increased with flock’s age (p < 0.001), but there was no significant treatment effect between 7 and 16 days of age (p > 0.05). At 19 days of age, the probability identifying oocysts in the fecal material was higher in TX1 compared to CONV (p = 0.03), but TX2 was not statistically different from TX1 and CONV (p > 0.05). Predicted oocysts per gram in CONV flocks were significantly lower from 22 to 34 days of age compared to TX1 and TX2 (p = 0.02), while there were no significant differences between TX1 and TX2 for the same time frame (p > 0.05). Lesion scoring of the gastro-intestinal system showed no differences for coccidiosis scores between TX1, TX2 and CONV (p > 0.05). No lesions of necrotic enteritis were observed in all flocks. In conclusion, it was possible to adequately control intestinal diseases and maintain zootechnical performances by relying exclusively on ionophores, when compared to broiler chicken flocks using standard shuttle programs with medically important antibiotics. The use of butyric acids showed no benefits and should not be included in full ionophore programs.

Key Words: Antibiotic reduction, Broiler, Intestinal health, Zootechnical performance, *Eimeria* spp.

55 A field study to evaluate the efficacy and safety of Panacur® AquaSol for use in drinking water for the treatment of chickens naturally infected with *Capillaria* spp. Rafael Chiummo*, Kevin Wilkinson1, Katharina Raue1, Christina Strube2, Emmanuel Thomas1, 2MSD Animal Health Innovation GmbH, Schwanheim, Germany, 1Institute for Parasitology, Centre for Infection Medicine, University of Veterinary Medicine Hannover, Hannover, Germany.

A field study was conducted in a laying hen farm in the Netherlands. The farm had 5810 free-ranged birds, that were 119 days of age. Treatment was administered for 5 consecutive days with fenbendazole solution for oral administration in drinking water (Panacur® AquaSol). Before treatment, 5 birds were randomly selected from the beginning, middle and end of the hen’s house for necropsy and evaluation of parasite infection burden. During necropsy, the intestines and caeca were shipped refrigerated (2-8 C) to the Institute for Parasitology of the University of Veterinary Medicine Hannover and processed. All the adult parasites, L4 and L5 stages were identified to genus and species. One fresh representative faecal sample was also collected for faecal egg counts using the McMaster technique to determine the *Capillaria* spp. egg count. Panacur® AquaSol was administered at the recommended dosage for *Capillaria* spp. of 2 mg fenbendazole/ kg body weight/ day for 5 consecutive days. To ensure administration of the correct dose, 100 birds (30 from the beginning, 40 from the middle and 30 from the end of the house) were weighed, to determine the average weight of the animals for dose calculation. Daily water consumption was measured over three days, prior to treatment. This information was used to calculate one-quarter of the average water daily consumption to be used with the medicated drinking water and ensure complete consumption. A dosing pump containing 110.2 mL of Panacur® AquaSol prediluted in 110.2 mL of water was used for treatment. On Study Day 14, 15 hens were euthanized and necropsied, and a representative faecal sample was collected following the same procedure of Study Day 0. Before treatment, the average number of *Capillaria* spp. pre-adult and adult stages per bird necropsied was 138.4, and the faecal egg count was 66.7 eggs per gram faeces. The most common species was *Capillaria obsignata*. The average number of *Capillaria* spp. per bird necropsied after treatment was 16.6, and the faecal egg count was 0. Treatment with Panacur® AquaSol resulted in the reduction of pre-adult and adult stages of *Capillaria* spp. from pre-treatment compared to post-treatment of 95.4%, and the efficacy based on results...
of faecal samplings pre- and post-treatment of 100%. No adverse events or abnormal clinical signs were observed during the study. There was no change in the mortality and laying rate either before or after treatment. This field study confirmed the safety and efficacy of Panacur® AquaSol, administered daily for 5 consecutive days at a dose rate of 2 mg fenbendazole/kg body weight per day, against natural infections with Capillaria spp. in laying hens.

Key Words: Capillaria, fenbendazole

56 Graded Eimeria infection regulates oxidative stress in broiler chickens and is strongly correlated with growth performance and gut health parameters. Po-Yun Teng 1, Yuqo H. Tompkins, Sudhir Yadav, Fernanda Castro, Aliberta L. Fuller, Woo Kim, Poultry Science, University of Georgia, Athens, Georgia, United States.

Coccidiosis is a parasitic disease caused by genus Eimeria, which induces malnutrition, gut damage, and high mortality. Our previous study has shown that the higher Eimeria challenge doses linearly regulated gut health parameters, including apparent ileal digestibility, gut permeability, and gut morphology. In the present study, an experiment was conducted to evaluate the effects of graded Eimeria challenge doses on gene expression of tight junctions and parameters of oxidative stress in the liver and serum. The correlation coefficients among doses, growth performance, and several parameters were calculated to understand the relationship among severity of Eimeria infection, gut health, and oxidative stress in broilers. The study included a control group and four challenge groups with different levels of Eimeria doses. A mixed Eimeriaspp., solution with 50,000 E. maxima, 50,000 E. tenella, and 250,000 E. acervulina per milliliter was prepared for the highest dose challenge treatment. The two-fold serial dilution was used to make the medium-high (25,000 E. maxima; 25,000 E. tenella; 125,000 E. acervulina), the medium-low (12,500 E. maxima; 12,500 E. tenella; 62,500 E. acervulina), and the lowest challenge dose (6,250 E. maxima; 6,250 E. tenella; 31,250 E. acervulina). A total of 360 thirteen-day-old male broilers were randomly allocated into five treatments with six replicated cages and challenged on the same day. The mucosa of jejunum and liver were collected on 6-d post-infection for tight junction proteins and antioxidant systems, respectively. The results showed that the gene expression of tight junction protein was linearly up-regulated with the increase in challenge doses (P<0.05). Moreover, Eimeria challenge increased superoxide dismutase (SOD) and decreased glutathione in the liver. However, only the lowest challenge dose upregulated gene expression of catalase (CAT) in the liver and serum. SOD and CAT did not linearly increase in response to the graded challenge doses. The results suggested that the severe Eimeria infection might damage and suppress the mechanisms of antioxidative systems in the liver of broilers. Furthermore, strong linear relationships were found between challenged doses and growth performance (r = -0.911), as well as the villi height to crypt depth ratio in the jejunum (r = -0.789). In conclusion, Eimeria infection increased oxidative stress in the liver of broilers. Moreover, the increase in Eimeria challenge dosage was strongly correlated with growth performance and parameters of intestinal integrity.

Key Words: Coccidiosis, Eimeria, Oxidative Stress, Gut health

57 A temporal investigation of host defense peptide expression and intestinal homeostasis in broiler chickens following a single infection with Eimeria acervulina. Sara Cloft 1, Kate Miska, Eric Wong, Animal and Poultry Sciences, Virginia Tech, Blacksburg, Virginia, United States, Animal Bioscience and Biotechnology Laboratory, USDA ARS, Beltsville, Maryland, United States.

Coccidiosis is a major economically detrimental disease worldwide in broiler chicken production and is characterized by consecutive infection and reinfection by multiple Eimeria species. Infection with the protozoan parasite Eimeria acervulina causes lesions in the duodenum. Previous research has identified changes in host defense peptides in response to Eimeria infections but focused on 7 days post-infection (dpi). Ascertainment of the temporal biological responses following a single Eimeria challenge is necessary to understanding disease progression and elucidating mechanisms behind recovery of the intestine. Under normal conditions the intestine exists in a state of continuous renewal; infection with Eimeria is thought to influence cellular renewal and alter intestinal homeostasis. Therefore, the objective of this study was to profile host defense peptide response to infection and identify genes involved in the return to intestinal homeostasis following infection with Eimeria. Heritage broilers were either infected with 100,000 E. acervulina oocysts or sham infected with sterile water. Segments of the duodenum were collected at 0, 3, 5, 7, 10, and 14 dpi for gene expression analysis using RT-qPCR. Seven avian β defensins (AvBD), Liver-enriched antimicrobial peptide-2 (LEAP2), Mucin-2 and 6 genes involved in maintaining intestinal homeostasis were evaluated using a 2-factor ANOVA and by infection status using a t-test. Mean separation via Tukey HSD test was conducted when significant (P ≤ 0.05) ANOVA results were obtained. Three genes displayed mRNA downregulation in infected birds between 3 to 7 dpi. In infected birds LEAP2 mRNA was decreased at 3, 5, 7, and 14 dpi (P ≤ 0.005). Mucin-2 mRNA abundance decreased at 3 dpi (P = 0.04) and 7 dpi (P = 0.07) and a proliferating cell marker, mKi67, was decreased at 5 dpi (P = 0.03) in infected birds. Changes in expression of a cluster of genes showed changes in gene expression in infected birds solely at 7 dpi. Infected birds at 7 dpi had decreased mRNA abundance for AvBD 1 and 6 (P < 0.03) but increased mRNA abundance for 3 genes involved in intestinal homeostasis: Collagen type 3α1, Wnt Inhibitory Factor-1, and Notch-1 (P ≤ 0.02). The Wnt and Notch pathways control the maintenance and differentiation of
intestinal stem cells, upregulation may indicate intestinal stem cell populations are working to replace damaged cells in the villi. No significant changes in gene expression were seen from at 10 and 14 dpi. The results in of this study showed that *E. acervulina* infection caused downregulation of host defense genes during infection (3 to 7 dpi). At 7 dpi there was upregulation of genes involved in intestinal homeostasis, indicating intestinal recovery.

**Key Words:** Coccidiosis, AvBD, LEAP2, Notch, Eimeria

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58 Evaluation of coccidial lesions in the ceca of broilers challenged with *Eimeria tenella* using digital image analysis. Ashlyn Snyder1, Kate Miska2, Cara Robison3, Shawna L. Weimer3, Animal and Avian Sciences, University of Maryland, College Park, Maryland, United States, USDA-ARS, Beltsville, Maryland, United States, Michigan State University, East Lansing, Michigan, United States.

Coccidiosis is one of the most prevalent gastrointestinal diseases and is responsible for significant economic losses in the broiler industry. To determine the pathogenicity of coccidia, intestinal lesions are typically categorically scored on a 0-4 scale by visual evaluation during bird necropsy. However, the inherent subjectivity of visual scoring has the potential to affect clinical diagnoses. To improve the accuracy and precision of lesion assessment, imaging technology has the potential to be used as an objective, decision-assistive tool. The objective of this study was to evaluate the utility of a digital image analysis method to quantify the severity of lesions in the ceca of broilers challenged with *E. tenella*. Male Ross 708 broilers (*N=288*) were raised in 24 battery cages and on day 21 half of the birds (*N=144*) were inoculated with 1 mL of 10^7 oocysts of *E. tenella* via oral gavage while the other half were sham infected with 1 mL water (control). Digital images of the ceca of 2 birds per cage from 12 cages were taken during visual scoring on days 3, 5, and 7 post-challenge (PC; *N=72*). Within each digital image, the length (cm), width (cm), total surface area (cm^2), and total lesion surface area (LA, cm^2) of the right and left ceca were measured in ImageJ. LA% was calculated through a red color threshold analysis on a standardized hue-saturation-brightness (HSB) scale. Measures of both ceca were averaged per bird and used for analysis. LA% was calculated as a percentage of the total surface area. Data were analyzed for the effects of treatment and days PC on image measures as well as for the relationship between lesion score and the calculated lesion area measures using mixed and linear regression models. Lesion scores were averaged and grouped into a three-point scale (0 = no lesions, 1 = mild lesions, 2 = moderate lesions). Challenge birds had wider ceca on PC days 5 and 7 compared to controls (*P ≤ 0.03; 1.79 and 1.89 cm vs. 1.47 and 1.66 cm, respectively*). Ceca lesions were the most severe for challenge birds on PC day 5 with the highest lesion scores (2), lesion area (1.65 cm^2) and percent lesion area (5.80%; *P ≤ 0.05*). The LA% for ceca with a lesion score of 2 had the highest LA% (1.84%) compared to scores of 1 (0.28%) and 0 (0.03%; *P < 0.0001*). The results from this study suggest that this digital image technology has the potential to be a useful diagnostic-active tool to determine the pathogenicity of coccidia.

**Key Words:** broiler, lesion, coccidiosis, digital image

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59 Immunomodulation using a dried egg product during a severe *Eimeria* challenge in broiler chickens. Muhammed Shameer Abdul Rasheed1, Utsav Tiwari1, Maci L. Oelschlager2, Brooke N. Smith1, Juliana Jepsersen1, Nanette Olmeda-Geniec2, Jeffery Escobar2, Ryan Dilger1, Animal Science, University of Illinois, Urbana, Illinois, United States, Elanco Animal Health, Greenfield, Indiana, United States.

Interleukin-10 (IL-10) secreted during *Eimeria* infection reduces the host’s ability to eliminate the parasite from gut epithelium by interfering with the Th1 response. Attenuation of IL-10 activity may allow the host to elicit a robust Th1 response via increased interferon-gamma production. An experiment was conducted to study the effects of feeding IL-10-neutralizing antibody from dried egg product (DEP) during a severe challenge following FDA CVM #217 protocol to test anticoocidial products in battery cages where birds were challenged with *E. acervulina* (Study 1) or *E. tenella* (Study 2). Evaluation in broilers was based on growth performance, immune responsivity, and gut health outcomes. A total of 720 male Ross 308 chicks were used in each study with 15 replicate cages of 12 birds and the following four treatments: sham-inoculated (uninfected) fed control diet, *Eimeria*-infected fed control diet, and *Eimeria*-infected fed control diet supplemented with DEP at 2 levels (165 or 287 in Study 1 and 143 or 287 U/tonne in Study 2). All infected groups received a single oral dose of either 200,000 *E. acervulina* (Study 1) or 80,000 *E. tenella* (Study 2) oocysts per bird at 12 d of age (i.e., d post-inoculation 0, DPI 0). A one-way ANOVA was performed on growth performance and immunophenotyping outcomes, repeated-measures ANOVA on blood chemistry and hematology outcomes, and a multinomial logistic regression was used for lesion scores. In both studies, DPI 0 to 7 weight gain, feed intake, and FCR were worse (P < 0.05) in all infected groups compared with the uninfected control group. Compared with the infected control group, DEP supplementation showed no differences on overall growth performance. Histopathology and lesion scores revealed severe damage to gut epithelium during *Eimeria* challenge, yet DEP supplementation did not improve outcomes in either category as well as oocyst shedding, hematological measurements, or serum chemistry. However, DEP supplementation improved (P < 0.05) percentage of circulating CD3+ cells 6 d after challenge infection in Study 2, thereby indicating an improvement in cellular immunity. Results showed DEP should not be considered as a coccidiostat. However, the increase in cellular immunity likely explains previous positive performance results in
broilers challenged with coccidiosis in field pen experimental studies.

Key Words: IL-10 neutralizing antibody, egg product, Eimeria, feed additive, broiler

60 Algae-based feed ingredient improves intestinal physiology and alters systemic immunity in broiler chickens during coccidiosis challenge. Krysten Fries-CraftGS*, Elizabeth A. Bobeck, Animal Science, Iowa State University, Ames, Iowa, United States.

The composition of nutritional and bioactive compounds in algae-based feed ingredients may influence broiler immunity and intestinal health to improve performance, especially under conditions of pathogenic or parasitic challenge. Therefore, algae inclusion may protect the avian intestine from coccidiosis-induced damage and alter the immune response; however, these outcomes are not widely studied and reported. The study objective was to examine the mechanism of a proprietary algae-based feed ingredient from ZIVO Biosciences Inc. (Keego Harbor, MI) on broiler intestinal health and splenic immune cell profiles during coccidiosis challenge. In total, 800 1d-old as-hatched Ross 308 broilers were housed in 40 pens allocated between 2 rooms (20 birds/pen). Birds were randomly assigned to a corn-soybean meal basal diet with or without 0.175% dietary algae inclusion, with or without coccidiosis challenge, resulting in 4 total treatments (10 pens/treatment). The 42d trial was divided into 14d starter, grower, and finisher periods with half of the birds orally inoculated with 10x Merck Coccivac®-B52 at d14. Five birds/treatment were euthanized for spleen, duodenum, and jejunum collection at d14 (baseline) and 3, 7, 14, and 28d post-inoculation (dpi). At 1, 3, 7, and 14dpi, 40 birds/treatment were orally gavaged with FITC-dextran, with blood collection 1hr post-gavage to detect serum fluorescence as a measure of intestinal integrity. Intestinal sections were formalin-fixed for microscopic measurement of villus height (VH) and crypt depth (CD) and splenic immune cell profiles were analyzed by multi-color flow cytometry. Data were analyzed using the MIXED procedure (SAS 9.4) with fixed effects of diet, health status, and the diet × health status interaction with significance at \( P \leq 0.05 \). Prior to challenge, feeding algae did not impact intestinal morphology, but increased CD3+ T-cells and the TCRγδ+ subpopulation by 29.6 and 1.93%, respectively, vs. control \( (P < 0.0001; P = 0.04) \). At 7dpi, both healthy and challenged algae-fed birds showed no differences in serum fluorescence or jejunal VH. In contrast, coccidia-inoculated birds on the control diet had a 1.5-fold increase in serum fluorescence \( (P = 0.04) \) and 64.9% greater jejunal VH compared to healthy control-fed birds \( (P < 0.0001) \). From 3-7dpi, coccidia-inoculated birds on the control diet showed a 19.6% reduction in splenic T-cells; whereas their algae-fed counterparts did not show a similar response until 14dpi \( (P = 0.02) \). These results suggest that feeding ZIVO algae ingredient may have beneficial impacts on the broiler intestine and immune response during coccidiosis challenge.

Key Words: broilers, coccidiosis, algae, immunity, intestinal integrity

61 Effects of antibiotic and probiotic feed additives on growth performance of Eimeria infected broilers. Linan JiaGS*, Wei Zhai, Poultry Science, Mississippi State University, Starkville, Mississippi, United States.

Effects of antibiotic and probiotic feed additives on growth performance of male and female broilers with or without cocci challenge were determined. A total of 672 male and 672 female Ross × Ross 708 one-day-old chicks were randomly assigned to 96 pens in 8 blocks. A randomized complete block design with \( 3 \times 2 \times 2 \) (3 diets × 2 cocci challenges × 2 sexes) factorial arrangement was used. The 3 diets included a control diet (corn-soybean meal basal diet), an antibiotic diet (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2 × 10^8 CFU Bacillus subtilis PB6 /kg feed). Birds were either gavaged with 1 ml 20 × cocci vaccine or 1 ml of distilled water on d 14. Growth performance data were analyzed using 3-way ANOVA. \( P \leq 0.05 \) was considered as significant. Mortality was not affected by cocci challenge or dietary additives, but female birds exhibited higher mortality from d 0-14 \( (P = 0.0229) \). On d 14, 29, 35, and 43, body weight (BW) of birds fed antibiotic diet was higher than that of those fed probiotic diet, with those fed control diet being intermediate \( (P = 0.0127, 0.0043, 0.0033, \text{and } 0.0523, \text{respectively}) \). The antibiotic additive also increased BW gain (BWG) from d 0-14 in comparison to probiotic diet and from d 14-29 in comparison to control diet and probiotic diet \( (P = 0.007 \text{ and } 0.013, \text{respectively}) \). Feed intake (FI) of birds fed antibiotic diet was higher than that of those fed probiotic diet, with those fed control diet being intermediate from d 0-14 and d 14-29 \( (P = 0.0033 \text{ and } 0.0453, \text{respectively}) \). However, antibiotic or probiotic supplementation did not affect feed conversion ratio (FCR) in any growth phases \( (P = 0.4449, 0.9920, 0.2348, \text{and } 0.1156, \text{respectively}) \). As expected, cocci challenge decreased BW on d 29 and 35 \( (P < 0.0001 \text{ and } P = 0.0315, \text{respectively}) \); decreased BWG and FI from d 14-29 \( (P < 0.0001 \text{ and } P = 0.0032, \text{respectively}) \); increased FCR from d 14-29 \( (P < 0.0001) \). However, cocci challenge increased BWG and FI from d 29-35 \( (P = 0.0001 \text{ and } 0.0007, \text{respectively}) \) and d 35-43 \( (P = 0.0023 \text{ and } P < 0.0001, \text{respectively}) \); decreased FCR from d 35-43 \( (P = 0.0527) \). From d 29-35, cocci challenge decreased FCR only in female birds \( (P = 0.0085) \) but not in male birds. Antibiotic increased BW and BWG at an early age due to increased FI, but antibiotic did not affect FCR. The growth restriction for the two weeks post-challenge and the subsequent growth acceleration may be due to the infection and recovery of coccidiosis. Antibiotic and probiotic supplementation did not alter the cocci impact on growth performance.

Key Words: Antibiotics, Probiotics, Coccidiosis, Growth Performance, Growth Rate
62 Changes in the ceca microbiota of broilers vaccinated for coccidiosis or supplemented with salinomycin. Catiane Orsó¹, Bruna S. Cony², Jessica P. Silva³, Julio C. V. Furtado², Bruna Schroeder², Ana P. G. Frazzon¹, Ines Andretta¹, Andréa M. L. Ribeiro², ¹Microbiology, UFRGS, Porto Alegre, RS, Brazil, ²Animal Science, UFRGS, Porto Alegre, RS, Brazil.

The coccidiosis vaccine induces intestinal mucogenesis and the reduction of nutrients digestibility and this may increase the proliferation of pathogenic microbiota. The objective was to characterize changes in the ceca microbiota of broilers vaccinated for coccidiosis. A total of 140 male 1-day-old broilers were separated in 2 groups (one vaccinated with a live vaccine and the other received salinomycin up to 35d), with 7 replicates of 10 birds raised in used wood shavings. At 28 days, the cecal content of one bird per replicate was collected. The V4 region 16S rRNA gene was amplified and the genetic sequencing was conducted by Illumina platform. The data were estimated using Microbiome and Phyloseq packages in R. Alpha diversity significance was estimated using non-parametric test Wilcoxon and Beta diversity by permutational multivariate analysis. Three major phyla (Bacteroidetes, Firmicutes, and Proteobacteria) were accounting for more than 98% of the total bacterial community. The vaccine did not reduce species richness and α-diversity (P>0.05) compared to Salinomycin group, but the richness distribution in this group was larger and more uniform than the vaccinated ones. Salinomycin group were showed enrichment of Bacteroidetes while Firmicutes were present in higher proportions in the vaccinated group. The Proteobacteria group appeared in a higher percentage in the vaccinated group and this phylum includes a wide variety of pathogenic bacteria. Bacteroides have a high relationship with themselves. Bacteroides also have a close relationship with the genus Alistipes, Lactobacillus, Subdoligranulum and Faecalibacterium. The genus Alistipes have a moderate relationship with Defluvialita and Oscillospira, and a stronger relation with Faecalibacterium. The genus of Ruminococcus has a stronger relationship with Shigella and Streptococcus. Shigella and Streptococcus are pathogenic bacteria, which cause harm to birds health. Clostridium genus was shown to be moderately related to Lactobacillus and Oscillospira and has a high correlation with Shigela, a pathogenic group. These relationships are still poorly understood and we cannot describe whether it is beneficial or maleficent. In general, the vaccinated group showed a lower percentage of phyla and genera that produce short-chain fatty acids and higher phylum Proteobacteria.

Key Words: Chicken, bacterial community, 16S, rRNA sequencing

63 Evaluation of the interaction between Eimeria adenoideae or E. tenella with Histomonas meleagridis in poult. Thaina L. Barroso¹,², John Barta¹,², Billy M. Hargis³, Christine Vuong¹, ¹Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, ²Ontario Veterinary College, Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada.

Infections with wild-type Histomonas meleagridis (HM), causing Histomoniasis, leads to high mortality rates in turkey and breeder broiler flocks. Eimeria tenella (Et) and E. adenoideae (Ead), etiological agents of Coccidiosis, are within another phylum of protozoa which are extremely common in poultry production. HM and Ead affect the ceca of turkeys, and Et infects the ceca of chickens and has been shown to cause cecal inflammation in turkeys. Previous research suggests that co-infection with HM and Et worsen the severity of Histomoniasis in broiler chickens and co-infection of Ead and HM in turkeys reduced severity. The objective of the present study was to evaluate if pre-inoculation with Ead or Et would impact the severity of Histomoniasis in turkeys. Day-of-hatch poult (n=32) were randomly assigned to five groups: negative control (NC); inoculation of 1x10⁶ HM only (HM); 9x10⁶ oocysts of Et and HM (Et+HM); and 500 (EadL+HM) or 2,500 oocysts of Ead (EadH+HM) and HM. Et and Ead were orally inoculated on day 15 and HM was intracoalocally inoculated on day 20. Poults were weighed on days 20 and 31 to record body weight gain (BWG). Mortalities and all surviving poult (d31) were lesion scored for hepatic and cecal Histomoniasis-related lesions (0-3 scale). Lesion scores were analyzed using PROC MIXED procedure (SAS 9.4). Mortality was compared using the chi-square test of independence. BWG was analyzed by ANOVA and means were separated with Duncan’s multiple range test. Significant differences are reported at P<0.05. BWG between the day of HM inoculation (d20) until the end of the experiment (d31) was lower for poult receiving HM only (126.7±28.07), compared to NC (451.3±15.39), Et+HM (261.3±39.35), EadL+HM (267.9±52.16) and EadH+HM (298.5±54.38). Poults receiving HM only had 33% mortality, not being different from Et+HM (15%). EadL+HM and EadH+HM experienced 2.5% mortality. Cecal and hepatic lesions were less severe in the poult that received either doses of Ead+HM, with cecal scores of 2.0±0.2 (EadL+HM) and 1.8±0.2 (EadH+HM), being significantly different from Et+HM (2.5±0.2) and HM (2.8±0.11). The same pattern was observed with liver lesions; 1.8±0.2 (EadL+HM), 1.7±0.2 (EadH+HM), 2.5±0.2 (Et+HM), and 2.8±0.1 (HM). A possible explanation for the findings of the present study is an activation of the cecal innate immunity by the E. adenoideae. Experiments investigating if a vaccination and consequent development of a solid acquired immune response towards E. adenoideae could provide an indirect and/or prolonged protection against Histomoniasis in turkeys are ongoing.

Key Words: Histomoniasis, Coccidiosis, turkey, immunity, ceca health

64 The importance of an in vivo model to confirm Clostridium perfringens strains pathogenicity. Nicolas
An effective vaccine against necrotic enteritis should preserve the chickens’ normal gut microbiota, including commensal *Clostridium perfringens* strains, while targeting pathogenic *C. perfringens* strains. Lack of predisposing factors for necrotic enteritis as well as in-feed administration of antibiotics are obstacles to the differentiation between pathogenic and commensal *C. perfringens* strains. Hypothesis and objectives: The presence of the NetB toxin has been highly correlated with *C. perfringens* enteritis. As the first step in the development of a vaccine against necrotic enteritis, the aim of this study was to preserve the chickens’ normal gut microbiota, including commensal *C. perfringens* strains, while targeting pathogenic *C. perfringens* strains.

**Material and methods:** Under general anesthesia, the jejunum of specific-pathogen-free chickens was surgically prepared in specific pathogen-free chickens and inoculated with selected *C. perfringens* strains to identify pathogenic strains. Multiple strains of different geographical origins (all from *C. perfringens*) were isolated from healthy chickens (3 NetB- and 6 NetB+) and presence/absence of netB while presence/absence of lesion was verified on HPS stained histological sections.

**Results:** Intestinal loops were surgically done in 14 anaesthetized chickens and inoculated with a total of 47 selected *C. perfringens* strains or a control (sterile broth). While the majority of NetB positive strains caused necrotic enteritis like lesions, 2 NetB+ strains caused no lesion. Interestingly, 9 NetB negative strains caused intestinal lesions. Finally, necrotic enteritis-like lesions were observed with 9 strains isolated from healthy chickens (3 NetB- and 6 NetB+). Conclusions: The loop model is necessary to confirm the pathogenicity of *C. perfringens* strains. Indeed, numerous NetB negative *C. perfringens* strains were shown to be pathogenic while a significant number of pathogenic strains were isolated from healthy chickens.

**Key Words:** in vivo model, Clostridium perfringens, pathogenicity, vaccine development, necrotic enteritis

65 Mammalian target of rapamycin signaling modulates chicken necrotic enteritis. Mohit Bansal1*,1, Ying Fu2, Ayidh Almansour2, Tahir Aleneji3, Mussie T. Abrah3, Danielle Graham1, Anamika Gupta1, Hong Wang4, Billy M. Hargis1, Xiaolun Sun3, Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, 3Cell and Molecular Biology & Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States.

Necrotic enteritis (NE) has reemerged as a prevalent chicken disease with huge losses every year after withdrawing antimicrobial growth promoters. Coccidia and *Clostridium perfringens* are the main pathogens responsible for NE. Very few effective antibiotic alternatives are available to prevent or treat NE. Here we hypothesized that the mammalian target of rapamycin (mTOR) inhibitor, rapamycin, could attenuate NE by blocking host proinflammatory response. To examine this hypothesis, broiler chicks were randomly allotted to one basal diet and six diets supplemented with four groups of rapamycin (0.075, 0.15, 0.3 0.45 mg/kg), 1.5 g/kg of deoxycholic acid (DCA), and 1.5 g/kg DCA plus 0.3 mg/kg rapamycin. Feed supplementation was started from d 17. Additional two groups of chickens were subcutaneously injected with rapamycin of 8 and 64 µg/kg BW starting from d 17. At 18 d, birds were orally infected with *Eimeria maxima* (15,000 sporulated oocysts/ bird) to induce coccidiosis. The birds were subsequently infected with 10⁸ CFU/bird of *C. perfringens* at d 23 and 24 to initiate NE. Growth performance of body weight gain (BWG) was measured at d 18, 23, and 25. The birds were euthanized at d 25. Ileum tissue and content were collected for *E. maxima* and *C. perfringens* colonization and histopathology analysis. The significant differences were calculated by one-way ANOVA and t-test with statistical significance when p ≤ 0.05. Notably, birds infected with *E. maxima* and *C. perfringens* developed acute NE and suffered severe growth performance reduction. Interestingly, any of the rapamycin treatment alone did not significantly inhibit the BWG loss induced by NE. During NE phase (d23-25), DCA attenuated the BWG loss (16 vs -14 g/day/bird) compared to NE group. Notably, DCA plus rapamycin trended to increase 80% of BWG compared to DCA alone (29 vs 16 g/day/bird, P = 0.08). DCA reduced both *C. perfringens* and *E. maxima* colonization compared to NE infected group (P < 0.05), while DCA plus rapamycin failed to reduce the pathogens. These findings indicate that reducing inflammation by rapamycin in conjunction with pathogen reduction by DCA attenuate chicken NE and its associated BWG reduction.

**Key Words:** Necrotic enteritis, Intestine inflammation, Bile acids, Rapamycin, Host response

66 Evaluation of plant polyphenols (Silvafeed® Nutri P Plant Bioactives) in the diet of broilers during a controlled necrotic enteritis challenge. Matthew K. Jones1*, Charles L. Hofacre1, Mariano Fernández Miyakawa1, Ernie E. Pierson4, Michele Battaglia1, Southern Poultry Research Group, Watkinsville, Georgia, United States, 1Consejo Nacional de Investigaciones Científicas y
Necrotic enteritis (NE) is an important disease to the broiler industry in the U.S. As broiler producers limit antibiotic use, they face the challenge of maintaining the birds’ enteric health and are looking for options to provide the birds a more stable platform to achieve this goal. Silvafeed® Nutri Plant Bioactives (Silvateam; NP) is a natural product extracted from woods of the European chestnut (Castanea sativa) and the Argentine quebracho (Schinopsis lorentzii). NP contains a combination of hydrolysable and condensed tannins which have been reported to have antimicrobial properties. To assess NP as a tool for NE control, a 28 day completely randomized block experiment with male Ross x Ross broiler chicks in a battery cage with four dietary (commercial type; crumbles) treatments: Treatment (TRT) 1. Negative control, no additive; 2. Treatment 1 + 55 g bacitracin methylene disalicylate (BMD)/MT; 3. Treatment 1 + 0.05% NP, and, 4. Treatment 1 + 0.10% NP. On day 14, all birds were inoculated (~5000 sporulated E. maxima oocysts/bird) then challenged with Clostridium perfringens (CP) (~1.0 x 10^6 CFU/mL) at 18, 19, and 20 days (Hofacre et al., 1998). Resulting TRT 1 necrotic enteritis mortality (16.0%) indicated a successful challenge. Treatment effects were observed on day 14 feed intake (kg/pen), mortality adjusted (MA), non-adjusted FCR and weight gain (kg/bird). TRT 4 birds had the highest intake (4.15%), TRT 2 and TRT 3 were intermediate (3.86% and 3.83%; respectively), and TRT 1 had the lowest 14-day feed intake (3.48%) (P < 0.05). Non-adjusted and MA-FCR, respectively, between TRT 1 (1.240^b and 1.230^b) and 2 (1.204^a and 1.202^b) were not different (P > 0.05). However; these groups were different than comparable values for TRT 3 (1.283^a and 1.278^a) and 4 (1.293^a and 1.293^a). On day 21, TRT 2, TRT 3, and TRT 4 had greater feed intake than the challenge control (TRT 1) (P < 0.05). 21-day MA-FCR in TRT 1 (1.321^a) and TRT 2 (1.290^b) were lower than those in TRT 3 (1.369^a) and 4 (1.387^a) (P < 0.05). Also, TRT 2 (0.693%) and TRT 4 (0.672%) elicited higher 21-day body weight gain (BGW) than TRT 1 (0.627%) whilst TRT 3 BWG (0.640%) was intermediate. Only BMD reduced NE associated mortality (0.0%) (P < 0.05). BWG at 28 days for TRTs 2, 3, and 4 were 1.20^a, 1.16^a, 1.20^a, respectively. These groups had higher 28 day BWG than TRT 1 (1.06^b) (P < 0.05). The feeding of diets with the combination of polyphenols present in NP during a severe NE challenge maintained weight gain at, and after, peak of challenge and may be a useful tool to lessen the impact of NE/CP challenge in broilers.

Key Words: Necrotic Enteritis, Broilers, Polyphenols, Tannins, Performance

67 Necrotic enteritis model to achieve mortality reflective of industry. Audrey F. Duff\textsuperscript{a,b}, Kaylin Chasser\textsuperscript{1}, Whitney Briggs\textsuperscript{1}, Johel Bielke\textsuperscript{1}, Shelby Ramírez\textsuperscript{2}, Antonia Tacconi\textsuperscript{3}, G. R. Murugesan\textsuperscript{2}, Lisa Bielke\textsuperscript{1}, \textit{Animal Sciences, The Ohio State University, Wooster, Ohio, United States, BIOMIN America Inc., Overland Park, Kansas, United States, BIOMIN Holding GmbH, Getzersdorf, Austria.}

Necrotic enteritis (NE) is an acute, multifactorial Clostridium perfringens (CP) infection characterized by severe necrosis of intestinal mucosa. Enteric damage sustained during NE infection contributes to significant economic losses associated with suppressed growth performance and increased flock mortality. Due to the impact of NE on bird welfare and profitability, development of non-antibiotic disease mitigation technologies and NE disease models remain essential for continued research and bird health within the industry. Here, Eimeria maxima M6 (M6) or Guelph (G) and bacterial challenges, Salmonella Typhimurium (ST) or E. coli LG (EC), were used alongside inoculation with various strains of CP (TX, 641, BB) to develop a model for NE reflective of industry conditions. A total of 270 day of hatch (DoH) broiler chicks were divided into one of six treatments: Uninoculated control (UC), M6+TX, ST+G+TX, EC+M6+TX, G+641, or G+BB. Chicks in ST+G+TX and EC+M6+TX were orally gavaged (OG) on DOH with 1x10^6 CFU/chick ST or 1x10^3 CFU/chick EC, respectively. On d14, all birds except UC were administered 6x10^6 oocysts/bird M6 or 8x10^4 oocysts/bird G via OG, and from d19-d21, 500mL of 1x10^8 CFU/mL of CP was added daily to feed respective to treatment. Body weights were measured on DoH, d14, and d22 to calculate weight gain (BGW) and %ChangeBGW relative to UC. Mortality was monitored for the duration of the experiment and all mortalities were necropsied to confirm NE status. All BWG data were analyzed via t-test against UC, and mortality data were analyzed relative to UC via Chi-squared analysis (\chi^2 > 3.841). All treatments exhibited significantly (p<0.05) less weight gain relative to UC during the challenge period (d14-d22), but the most dramatic impact was observed in G+641 and G+BB which exhibited a 42.02% and 46.10% reduction in BWG relative to UC, respectively. As expected, mortalities were predominately observed throughout the NE period (d19-d22). Inoculation with EC increased mortality to 6.67% compared with M6 alone (2.22%). Inoculation with ST+G+TX did not produce any mortality; however, G+641 and G+BB increased mortality to 26.67% (\chi^2 > 20.00) and 6.67% respectively. Thus, the strain of CP used and the introduction of EC or ST can have a large influence on the mortality. However, all challenge models produced significant weight reduction compared with UC. Characterizing NE models based on performance and mortality outcomes that reflect those in industry create more opportunities to test different mitigation strategies for NE.

Key Words: Necrotic enteritis, Mortality, Clostridium perfringens, Broiler, Model

68 Evaluation of bile acids against chicken necrotic enteritis. Mohit Bansal\textsuperscript{a,b}, Ying Fu\textsuperscript{1}, Ayidh Almansour\textsuperscript{2}, Mussie T. Abraha\textsuperscript{1}, Tahir Aleneiji\textsuperscript{1}, Danielle Graham\textsuperscript{1}, Anamika Gupta\textsuperscript{1}, Hong Wang\textsuperscript{1}, Rohana Liyanage\textsuperscript{1}, Billy M.
Necrotic enteritis (NE), mainly induced by pathogens of Clostridium perfringens and coccidia, causes huge economic losses in the poultry industry. The withdrawal of antimicrobial growth promoter is one of the main factors for the reemergence of NE. This study was to investigate the impact of various bile acids on alleviation of chicken NE. Day-old broiler chicks were randomly assigned to 7 groups of diets supplemented with 0 (basal diet), 1% commercial bile, 1.5% deoxycholic acid (DCA), 1.5% lithocholic acid (LCA) and 4000 ppm of chicken bile. The birds were challenged with Eimeria maxima (15,000 oocysts/bird) at d 18 and C. perfringens (10^7 CFU/bird/day) at d 23 and d 24 to induce NE. Birds were euthanized at d 25 and ileal tissue was collected for histopathology and mRNA accumulation analysis. Ileum content samples were collected for bile acid quantification using LC-MS/MS. The significant differences were analyzed by one-way ANOVA and t-test with statistical significance when p ≤ 0.05. Notably, birds infected with E. maxima and C. perfringens developed acute NE and suffered severe growth performance reduction of daily body weight gain (BWG) during the NE phase compared to noninfected birds (-14 vs. 56 g/day/bird, P < 0.001). Both commercial bile and chicken bile failed to attenuate body weight gain loss (-6 and -15 vs -14 g/day/bird) compared to NE, respectively. Interestingly, DCA at 1.5 g/kg in feed alleviated the NE-induced BWG loss (16 vs -14 g/day/bird, P < 0.006) compared to NE birds. Molecular analysis showed that C. perfringens colonization increased significantly by 2 logs in NE infected birds (6.36 vs 4.12 logs, P = 0.004) compared to noninfected birds. C. perfringens colonization was decreased significantly in the DCA group (5.31 vs 6.36 logs, P = 0.04) compared to NE infected group. NE infection modulated the major bile acids (CA and CDCA) composition in ileum content. Dietary DCA, LCA, commercial bile, and chicken bile significantly increased the T/DCA (4410 vs 60 nmol/gram, P = 0.007), total LCA (885 vs 2.5 nmol/gram, P = 0.015), total T/G/CA (606 vs 3036 nmol/gram, P < 0.001), and total T/G/CDCA (1267 vs 2805 nmol/gram, P = 0.09) in ileum content, respectively. The results showed that dietary secondary bile acids DCA, but not other bile acids, reduces C. perfringens colonization in NE infected birds.

Key Words: Necrotic enteritis, Intestine inflammation, Bile acids, Microbial metabolites, C. perfringens

70 Mucosal immunity of broilers vaccinated with an oral polyanhydride-nanoparticle based Clostridium perfringens vaccine expressing Salmonella enterica flagella. Nour Ramadan*1,2, Gabriel Akerele1, Sankar Renu1, Renukaradhya Gourapura1, Ramesh Selvaraj1, Poultry Science, University of Georgia, Athens, Georgia, United States, 2Food Animal Health, Ohio state university, Wooster, Ohio, United States.

Clostridium perfringens is one of the predisposing factors in causing necrotic enteritis in poultry. Two-experiments were performed to analyze the immunogenicity of two synthesized oral Clostridium perfringens polyanhydride-
based nanoparticle vaccines. Two polyanhydride nanoparticle vaccines were synthesized, a *C. perfringens* toxin (PN) and a *C. perfringens* toxoid (PT). Both vaccines were surface coated with *Salmonella* flagella proteins. In experiment I, 90 one-day-old male Cobb-500 broilers were orally gavaged with PBS (control) or 50µg of PN or PT vaccines in six replications (n=6). Boosters with same vaccine dosage were administered at 3-, 7- and 14-days post-hatch. In experiment II, 1-day-old male Cobb-500 broilers were orally gavaged with 0 (control) or 50µg of PN vaccine in six replications. Boosters with same vaccine dosage were administered at 3-, 7- and 14-days post-hatch. Performance parameters were measured at d0, 7, 14, 21, and 28. Blood and bile were collected at d3, 10, 17, 21, and 28 for seroconversion. Spleen samples were collected d17 for analyzing T cell proliferation. Sera and spleen samples collected on d17 and d21 were used to assess neutralizing antibodies content. Ceacal tonsils and ceacal contents were collected at d17 for flow cytometry and qPCR DNA quantification, respectively. Data was analyzed using one-way ANOVA or parametric t-test followed by Tukey’s HSD test for mean separation. There were no significant differences in BWG and FCR between treatments in experiment I, there were no differences in anti-CP specific IgA and IgY antibodies in experiment I and II. In experiment I, serum of birds in the PN and PT groups had significantly higher percentage (P<0.01) of neutralizing antibodies compared to that in the control group. PN and PT candidate vaccines, administered through oral gavage, had no adverse effects on bird performance and were immunogenic and can potentially be used to control necrotic enteritis in poultry.

**Key Words:** Clostridium perfringens, Vaccine, Immunology, Nanoparticle

71 Direct evidence for the production of an adhesive pilus by *Clostridium perfringens* that is required for the pathogenesis of necrotic enteritis in poultry. Dion Lepp1, 2; Yuanyuan Zhou1, 3, Shivani Ojha1, Imran Mehdizadeh Gohari1, Jason Carere1, Chengbo Yang1, John F. Prescott1, Joshua Gong1, 1Guelph Research and Development Centre, Agriculture and Agri-Food Canada, Guelph, Ontario, Canada, 2Dept of Pathobiology, University of Guelph, Guelph, Ontario, Canada, 3Dept of Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada.

NetB-positive Type G *Clostridium perfringens* strains cause necrotic enteritis (NE) in poultry, an economically important disease that is the major target of in-feed antibiotics. NE is a multifactorial disease, and while the NetB toxin is critical for its development, additional virulence factors are also involved. Histological analysis of the characteristic small intestinal lesions produced by NE typically reveals large numbers of *C. perfringens* cells adhering to the necrotic tissue, indicating that adherence is a key step in pathogenesis. We previously identified a *C. perfringens* chromosomal locus (VR-10B) associated with disease-causing strains that is predicted to encode an adhesive pilus; however, this has yet to be confirmed experimentally. This locus was recently shown to be involved in binding to collagen and required for NE pathogenesis. In the current study, we sought to provide direct evidence for the production of a sortase-dependant pilus by *C. perfringens*. In order to visualize pilus structures in virulent *C. perfringens* strain CP1, rabbit polyclonal antibodies were raised against the three predicted pilin subunits encoded by VR-10B (*cnaA, fimA, and fimB*), and used for immuno-gold labelling and transmission electron microscopy (TEM) of bacterial cells. Immuno-labelled filamentous structures were observed in wild-type CP1 extending from the cell surface, but were absent from isogenic pilin-null mutant strains. Pilus production was also demonstrated by Western blotting of cell surface proteins, in which CP1, but not the null mutant strains, produced a high molecular weight ladder-like pattern characteristic of a pilus polymer. Binding to collagen types I, II and IV was significantly reduced (Tukey’s; p<0.01) in all three pilin mutants compared to CP1, and could be specifically blocked by anti-CnaA and -FimA antibodies, indicating that these pilins participate in adherence. Furthermore, *fimA* and *fimb* null mutants were assessed in an *in vivo* chicken NE challenge model and were both severely attenuated in their ability to cause disease (mean NE lesion score: CP1=2.6, CP1/fimA=0.43, CP1/fimb=0; Tukey’s p<0.001 compared to CP1). To our knowledge, this is the first report of the production of a sortase-dependant pilus in *C. perfringens*. Together, these results provide direct evidence that the VR-10B locus encodes a functional pilus, and confirm that this adhesive structure specifically mediates binding to collagen and is necessary for NE pathogenesis.

**Key Words:** Clostridium perfringens, Necrotic enteritis, Pilus, Adherence, pathogenesis

72 PilR/PilS two-component and Agr-like quorum sensing systems regulate pilin production and binding of *Clostridium perfringens* to collagen. Yuanyuan Zhou1, 2, 2, Dion Lepp1, Jason Carere1, Hai Yu1, Chengbo Yang1, Joshua Gong1, 1Guelph Research and Development Centre, Agriculture and Agri-Food Canada, Guelph, Ontario, Canada, 2Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada.

*Clostridium perfringens* type G isolates cause necrotic enteritis (NE) in poultry. Their pilus plays a role in adhesion and is important in pathogenesis. The pilus in these isolates consists of three pilins (*CnaA, FimA, FimB*) encoded by three genes that belong to the VR-10B allele of the VR-10
locus. There are two genes downstream of the pilin genes encoding a two-component regulatory system (PilR/PilS). However, the regulatory function of PilR/PilS remains to be determined. The current study has investigated the function of PilR/PilS in the regulation of pilin production and binding to collagen by comparison of isogenic pilR-null and complemented strains with the parent strain CP1. In addition, the role of the Agr-like QS system in regulating pilin production and binding has been examined by comparing isogenic agrB-null and virR-null mutants and their respective complemented strains with CP1. Western blot analyses showed no detectable pilus production in mutant pilR, while production by its complemented strain was similar to wild-type levels. In contrast, pilus production was the same or higher in the agrB and virR mutants, but reduced in their respective complements compared with CP1. Similarly, no pilus production was detected in the pilin mutant strains (cnaA, fimA, or fimB). These observations were supported by the binding results to collagen of mutants pilR, agrB, and virR and their complemented strains, with CP1 as a reference. The pilR mutant showed significantly less binding than CP1 (p ≤ 0.05) to most collagen types, but its complement was similar to the parent strain in binding (p > 0.05). In contrast, binding of agrB and virR mutants to collagen (types I–V) showed no significant changes compared with CP1 (p > 0.05), whereas their complemented strains had significantly reduced binding to collagen types I–IV (p ≤ 0.05). To confirm that the collagen binding in the agrB mutant was specifically mediated by the pilus, and not due to the expression of other factors regulated by the Agr-like QS system, binding of CP1 and mutant agrB to type I and IV collagen was examined in the presence of anti-CnaA, FimA, or FimB antibodies. Antibodies against both CnaA and FimA blocked the binding of CP1 and the mutant in a dose-dependent manner. However, the antibody against FimB showed no blocking of binding. These data suggest that the PilR/PilS two-component system positively regulates pilin production, while the Agr-like QS system may negatively regulate pilin production in C. perfringens.

Key Words: Pilus production/regulation, Binding, PilR/PilS, Agr-like quorum sensing, Clostridium perfringens

73 Not Presented.

74 Not Presented.

75 Not Presented.

76 The role of folic acid on the antiviral innate immune pathways in chicken B lymphocytes infected with IBDV. Santiago Uribe-Diaz*,1, Alexander Yitbarek2, Daniela Losada-Medina1, Marya Ahmed1, Juan Carlos Rodriguez-Lecompte1, 1University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada, 1University of Guelph, Guelph, Ontario, Canada.

This study aimed to assess the effects of FA supplementation on the antiviral immune pathways in chicken B lymphocytes infected with IBDV. Methods: Chicken B lymphocytes (DT-40) cell line were cultured in triplicate at a concentration of 5x105 cells / well in plates of 24 wells. B cells were divided into three groups: 1: No virus, no FA; 2: Virus, no FA; 3: Virus + FA at a concentration of 3.96 mM. After 12 hours of culture, cells were inoculated with a modified mild live vaccine of IBDV (UNIVAX-BD®) at 1 multiplicity of infection (MOI=1) (Groups 2 and 3). Followed by 1 hour of virus adsorption, cells received media supplemented with 3.96 mM of FA (Group 3), or medium alone (groups 1 and 2). Samples were collected at 0, 3, 6, 12, 24, and 36 hours post-infection (hpi) and mRNA expression levels were evaluated for the reduced-folate carrier (RFC), toll-like receptors (TLR) 3 and 21, MDA5, TRIF, MyD88, IRF-7, IFN-ß, OAS, PKR and viperin by RT-PCR. This experiment was repeated three times. 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 three treatment groups were performed using Tukey’s adjustment. Results: Viral load increased gradually from time 0 post-infection (group 2 and 3) and reached its highest peak at 6 hpi in group 2. In group 3, viral load peaked at 24 hpi. Besides, at 36 hpi a downregulation of viral load was observed in group 3 (P<0.05) compared to group 2. Reduce folate carrier gene expression was upregulated (P<0.05) at 12 hpi in group 3. In the same group, a downregulation of TLR-3 was observed at all time points (P<0.05), except at 12 hpi (P>0.05). There was a significant upregulation (p<0.05) of MDA5 in group 3 at 12 and 24 hpi; in contrast, MDA5 was downregulated (P<0.05) at 3 and 6 hpi. Expressions of TRIF and MyD88 genes were significantly upregulated at 12 and 24 hpi (P<0.05) in group 3 when compared to group 2. Although there were not significant differences (P>0.05) in IFN-ß, there was a significant difference in OAS at all time points post-infection, however, PKR showed no differences (P>0.05) except at 24 hpi. Viperin was significantly upregulated (P<0.05) in group 3 at 0, 3, and 24 hpi while it was significantly downregulated at 6 hpi when compared to the control (P<0.05). Conclusion: A time-course gene expression analysis demonstrated the effect of FA supplementation on chicken B lymphocytes, focus on antiviral innate immune response during an IBDV infection. According to our results, FA can delay IBDV infection in B lymphocytes. Moreover, FA can modulate the antiviral cellular innate immune pathways involved in IBDV infection.

Key Words: Innate immunity, B lymphocyte, IBDV, Folic acid, Gene expression

77 Environment affects the intestinal microbiota of broilers chicks. Martine Boulianne*, Laura Franco1, Marcio Costa1, 1Pathology and microbiology, Faculté de médecine vétérinaire, Université de Montréal, St.Hyacinthe, Quebec,
Dynamics of bacterial population transfer through the various steps of the modern poultry production chain are poorly known. Eggs are quickly collected, cooled and stored at the breeder barns before shipment to the hatchery to be stored, incubated, vaccinated at transfer then hatched in large groups. Yet the origin of the chick intestinal microbiota is believed to be mostly influenced by their parents. Our objectives were to further explore the role of broiler breeder gut microbiota on their progeny intestinal microbiota and evaluate factors influencing intestinal colonization of broiler chicks. Fecal samples (n=8 per flock) were collected from two different breeder flocks located on the same farm, twice, two weeks apart. In addition, cecal samples were collected from chicks (n=8 per farm) originating from one of these breeder flocks, hatched on the same day, but placed onto two different broiler farms. Cecal content was collected at necropsy in two-day old chicks. The V4 region of the 16S rRNA gene was amplified by PCR and sequenced with an Illumina Miseq. The software mothur was used for bioinformatic analysis. Analysis of molecular variance (AMOVA) was used to compare microbiota profiles (beta diversity analysis) between the different ages (breeders versus chicks), breeder barn and sampling time (2 weeks apart), and between chicks destination farms. As expected, bacterial communities differed according to age (breeders versus chicks, P<0.001). There was no significant difference between the two breeder flocks nor overtime within the same flock (all P>0.05).

Interestingly, although originated from the same breeder farm, chicks allocated in two different farms had different bacterial microbiota (P<0.001). We concluded that the microbiota of adult broiler breeders was stable over a two week period and similar for flocks located on the same premises. The destination farm where chicks were housed was a major factor of influence on their intestinal colonization, indicating that environmental factors strongly contribute to the microbiota composition. Thus, methods of microbiota manipulation (e.g. environmental probiotics) deserve further investigation.

**Key Words:** microbiota, 16S sequencing, intestinal colonization, poultry, animal production

78 Effects of xylanase and xylooligosaccharides supplementation on productive performance and gut health variables of broilers. Amit K. Singh*, Birendra Mishra1, Mike Bedford2, Rajesh Jha1, 1Human Nutrition, Food and Animal Sciences, University of Hawaii at Manoa, Honolulu, Hawaii, United States, 2AB Vista Feed Ingredients, Marlborough, Wiltshire, United Kingdom.

The aim of this study was to evaluate the effects of xylanase and xylooligosaccharides (XOS) supplementation in corn-soybean meal (SBM) based diet on growth performance and gut health parameters of broilers. A total of 288 day-old chicks (Cobb 500) were equally distributed to 36 floor pens (8 birds/pen) and the pens were randomly allocated to 9 dietary treatments (n=4) in 3×3 factorial arrangement. The treatments included: combination of 3 levels of xylanase (0%, 0.005% and 0.01% Econase XT) and 3 levels of XOS (0%, 0.005% and 0.01%) added to basal diets formulated in three phases (starter, d 0-14; grower, d 15-28; finisher, d 29-42). The birds were raised for 42 d under standard husbandry practices and were fed mash feed ad libitum. The feed intake and body weight were recorded weekly to determine feed conversion ratio and growth performance. On d 42 ileal samples were collected for histomorphometric and gene expression analysis, whereas cecal digesta was collected for short chain fatty acids (SCFA) and microbiota determination. Xylanase supplementation significantly increased the average total body weight (ATW, 2940 & 2932 vs 2760 g) of broilers (P <0.01). Xylanase increased (P <0.05) the average daily gain (ADG) in the finisher and total period, but no difference was observed in ATW and ADG in response to XOS. The effect of 0.005% xylanase on ADG was not different from 0.01% xylanase and control (P>0.05). Neither xylanase nor XOS affected average daily feed intake, feed conversion ratio, and ileal morphology of villus height and crypt depth (P>0.05). However, xylanase had a trend (P =0.097) on villus height to crypt depth ratio. The treatment containing 0.01% XOS without xylanase exhibited a higher level of IL-10 (a marker of anti-inflammatory cytokine) and IL-4 (a T-cell differentiation cytokine) genes compared with control (P <0.05). The production of acetate was increased by both xylanase (P<0.01) and XOS (P<0.05), but their additive effect was not evident. Xylanase also increased the production of total SCFA (P <0.01), while XOS exhibited a trend (P =0.052). 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, 0.01% xylanase increased the mean proportion of family Ruminococcaceae compared with no xylanase groups (P <0.01). The results infer that xylanase can improve the growth performance and cecal fermentation in broilers when supplemented in corn-SBM diets along with XOS, but the effects may not always translate into improved feed efficiency or better mucosal absorptive capacity.

**Key Words:** broilers, gut microbiota, short chain fatty acids, xylanase, xylooligosaccharides

79 Effect of NSPase enzyme and residual fiber from digested feed on cecal short chain fatty acids production and cecal microbiota diversity in broilers, studied in vitro. Amit K. Singh*, Joshua Legaspi1,2, Timothy Park1,2, Kabi Neupane2, Rajesh Jha1, 1Human Nutrition, Food and Animal Sciences, University of Hawaii at Manoa, Honolulu, Hawaii, United States, 2Leeward Community College, University of Hawaii, Pearl City, Hawaii, United States.

It has been reported that feed enzymes and dietary fiber can modify the gut microbiota in chickens. However, it has not been understood if exogenous NSPase enzyme could act on
the residual fiber passed to hind gut to provide resources for fermentation and modification of cecal microbiota in broilers. This study evaluated the effect of in vitro fermentation of ileal residue of fibrous diets without or with xylanase enzyme on the production of cecal short chain fatty acids (SCFA) and variation in cecal microbiota profile in broilers. The ileal residue contained fermentable carbohydrates which was obtained after two steps in vitro enzymatic digestion. The experiment consisted of a 6-treatments combination of 0%, 5% & 10% wheat bran (WB) without or with xylanase (0.01% Econase XT) in 3×2 factorial arrangement. Inulin and blank were used as experimental controls. After 48 hours of incubation at 39 ± 0.5 ºC, the supernatant was aspirated for SCFA determination and the pellet was used to extract microbial DNA. The SCFA was determined using gas chromatography and trimethyl acetic acid was used as an internal standard. The PCR product was used for microbial genome sequencing using Next Generation Sequencing technique. The data were analyzed using GLM procedure of SAS for SCFA and T-test was employed for bacterial abundance, and the significance was declared at P <0.05. Neither the level of WB in feed, nor the enzyme affected the cecal SCFA production (P >0.05). Likewise, the treatments did not modify (P >0.05) the overall alpha or beta diversity of cecal microbiota. However, the count of bacterial abundance revealed that xylanase increased the difference in the mean proportion of orders bacteriodales and lactobacillales compared with no xylanase groups (P <0.05). In conclusion, the feed residues of all treatments were utilized similarly by the cecal microbes for SCFA production and their balance in cecal colonization was modified by the addition of xylanase enzyme. Hence, this study reveals that slight variation in type of fiber entering the ceca and fiber degrading enzymes can shift the diversity of cecal microbiota in broilers, but it might not produce a significant change in fermentation metabolites. However, early and periodical sampling from in vitro fermentation could provide more information on the production of fermentation metabolites.

Key Words: fermentation, in vitro, microbiota, short chain fatty acids, xylanase

80 Effects of in ovo inoculation of chicken embryos with chitooligosaccharide and chlorella polysaccharide on the gut health parameters of broiler chickens. Jiachao Zhang1,2, Kun Cai2, Rajeev K. Mishra1, Rajesh Jha*1, 1Human Nutrition, Food and Animal Sciences, University of Hawaii at Manoa, Honolulu, Hawaii, United States, 2College of Food Science and Technology, Hainan University, Haikou, Hainan, China.

The chitooligosaccharide (COS) and chlorella polysaccharide (CPS) have been widely used as feed supplements in the livestock and poultry industries for improving the growth performance and immunity. However, the benefits of these prebiotics in chicken early nutrition is unknown. This study evaluated the impacts of COS and CPS feeding in ovo on the cecal metabolites and the intestinal microbiome of chicken. A total of 240 fertile eggs were divided into 6 treatment groups (n=4; 10 eggs/replicate): 1) no-injection (NI) control, 2) normal-saline (NS) control, 3) COS 5 mg, 4) COS 20 mg, 5) CPS 5 mg, and 6) CPS 20 mg injection. On d 12.5 of egg incubation, test substrate (except for NS) dissolved in 0.5 ml of 0.85% NS was injected in the amniotic sac of eggs in respective treatments. The chicks were raised for 21 d post-hatch under standard husbandry practices and were fed a commercial broilers diet. The feed intake and body weight were recorded weekly. On d 21, cecal digesta was collected to determine short-chain fatty acids (SCFA) using gas chromatography and microbiota using shotgun metagenomic sequencing. On d 3, there was no difference (P>0.05) in cecal microbial composition among different groups. However, with the increase in age, the cecal microbiota of each group was distinct with respect to organismal structure. At species level, the polysaccharide utilizing bacteria including Lactobacillus johnsonii, Bacteroides coprocola and Bacteroides salanitronis were higher (P<0.05) in the COS group, while the relative abundance of some pathogenic bacteria like Clostridium perfringens, Klebsiella unclassified, Shigella boydii and Shigella sonnei were lower (P<0.05) than the control and CPS groups. At the functional level, the pathways of gluconeogenesis, L-isoleucine degradation, L-histidine biosynthesis and fatty acid biosynthesis were enriched in the COS group. The propionic acid was significantly higher (P<0.05) in the cecum of the COS 5 mg group. At last, a network based on the correlation between the COS and other factors was constructed to illuminate the potential action mechanism of the COS in chicken early nutrition. In conclusion, in ovo inoculation of COS 5 mg showed positive effects on the cecal microbiota and propionic acid, thus can be used as in ovo feeding to modulate gut health of broiler chickens.

Key Words: In ovo feeding, Chitooligosaccharide, chlorella polysaccharide, intestinal microbiota, metabolic pathways

81 Probiotic cocktail accelerates the succession of ileal microbiota and trajectory of intestinal development in broiler chickens. Dazhi Tang**, Bochen Song, Tahir Mahmood, Peng Li, Yuming Guo, College of Animal Science and Technology, China Agriculture University, Beijing, China.

The gut microbiota plays an important role in the establishment and maintenance of host mucosal homeostasis. Nevertheless, the underlying mechanism of mutual response between microorganisms and small intestine is not fully elucidated. In this study, we explored the putative mechanism of probiotic cocktail on the succession pattern of microbiome and the effects on remodeling of ileal development in chicken. A total of 240 male Ross 308 broilers were randomly divided into the control group (CON) and the probiotic cocktail (including
Bacillus subtilis, Bacillus velezensis, Bacillus licheniformis, and Bacillus amylobiofaciens) supplemental group (PRO), with 10 replicate pens in each group. The experimental period lasted for 21 days. One broiler was selected from each replicate pen of both treatment groups at 7, 14, and 21 days, respectively, to collect ileal digesta (Lu), mucosa (Mu), and intestinal tissue samples. High-throughput sequencing and bioinformatics analysis of the microbiome and intestinal transcriptome were used to reveal the succession of microbial communities in the ileum and the mechanism of differential gene expression during intestinal development. Student's t-tests and non-parametric tests were used for statistical analysis. At 21 days old, body weight and microvilli length of the ileal epithelial cells were significantly better in PRO than in CON (p < 0.05). The dominant microorganism of Lu and Mu was Lactobacillus and Candidatus Arthromitus in both groups, respectively, and the relative abundance of two species demonstrated opposite succession patterns in different habitats. At 14 days old, Candidatus Arthromitus became marker species of Lu in both groups. While Bacteroidaceae and Ruminococcaceae of Mu were used as marker species in PRO. PICRUSt results demonstrated the metabolic pathways of beneficial products such as folic and propionate were increased significantly with broiler growth in Pro (q = 9.39e-03 and q = 1.30e-02). Meanwhile, the trace-source analysis found that the probiotic cocktail caused the main bacterial constituent in Lu to alter from 21-day-old Mu sources to 7-day-old Lu sources. Temporal analysis of ileal transcriptome exhibited two clusters in CON, where immune molecular regulations were significantly activated and developmental signaling pathways were significantly repressed. The probiotic cocktail reversed the oxidative phosphorylation and developmental pathways. Thus, it can be concluded that probiotics may achieve growth-promoting effects by facilitating the succession of intestinal microbial communities and reshaping ileal gene expressions in the host.

Key Words: cocktail probiotics, microbial community, ileal transcriptome, intestinal development, broiler chickens

82 Effects of dietary supplementation of probiotics on immune function and ileal microorganisms in caged broiler chicks. Bochen Song1,2,*, Dazhi Tang1, Hao Fan1, Liping Gan1, Shaojia Yan1, Guang Li1, Peng Li1, Shengyu Zhou1, Yuming Guo1, 1College of Animal Science and Technology, China Agricultural University, Beijing, China, 2College of Animal Science and Technology, China Agriculture University, Beijing, China.

The purpose of this experiment was to study the effects of dietary supplementation of probiotics on immune function, and ileal microorganisms of broiler chicks kept in laminated cages, and provide a scientific basis for the regulation of immune nutrition. A total of 200 male 1-day-old Arbor Acres broiler chicks were randomly assigned to 2 dietary treatment groups. Each treatment group consisted of 10 replicate cages with 10 birds per replicate pen. Water and feed were provided ad libitum. Dietary treatments consisted of a control basal diet without compound probiotics supplementation, and basal diets containing 2 × 10^9 CFU/kg compound probiotics. Broiler chicks were sampled after euthanasia on day 6, 13, 20, 27, and 34. The concentrations of cytokines in serum samples were determined with commercially available chicken cytokine ELISA kits. The proportion of immune cells was assayed through flow cytometry. A 3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide assay was used to determine the peripheral blood lymphocyte proliferation response. The gene expression was assayed through RT-PCR. The ileum microbiota was assayed through 16SrRNA sequencing technology. 16S rRNA sequencing of compound probiotic additive showed that it was mainly composed of Bacillus (62.228%), Lactococcus (3.213%), Lactobacillus (3.813%), Enterococcus (2.562%), Pediococcus (2.040%), Streptococcus (1.014%) and others. The addition of probiotics significantly increased monocyte phagocytic activity, NO, C3, C4, lysozyme activity, T cell ratio, Th cell ratio, CD4 / CD8, T cell proliferation activity, IFN-γ, B cell proliferation activity, IgG, IgA and IgM in the peripheral blood (P < 0.05). Moreover, probiotics increased mRNA levels of TLR2, TLR4, TNF-α, IL-6, IFN-γ and IL-4 in the spleen (P < 0.05). Furthermore, probiotics increased mRNA levels of MHC II, TLR4, IL-8, IFN-γ / IL-4, pIgR, and IgA in the ileum (P < 0.05), and sIgA level in the ileal mucosa (P < 0.05). For the ileum microorganisms, probiotics significantly increased the α diversity of ileum microorganisms on day 1-20 broiler chicks (P < 0.05), and increased proportion of relative abundance of Enterococcus, Pediococcus and Streptococcus (P < 0.05), these three significantly increased microorganisms may come from the compound probiotic additive. It could be concluded that dietary supplementation with probiotics significantly improved broiler immune function, and colonized the ileum with Enterococcus, Pediococcus, and Streptococcus. Peptidoglycans on these three Gram-positive cocci may stimulate the TLR2-NFκB signaling pathway of phagocytes in the ileum, which leads to a stronger immune function.

Key Words: Broiler, Probiotics, Immunity, Intestinal microorganisms, Cage rearing

83 Efficacy of Pleurotus ostreatus (oyster mushroom) extracts and isolated proteins in the production profile, oxidative stress and immunity of the broiler birds. Haseeb Anwar*, Ghulam Hussain, Intiaz Mustafa, Physiology, Government College University, Faisalabad, Punjab, Pakistan.

Pleurotus ostreatus (Oyster) is a worldwide used mushroom, known to have a wide range of nutrients including dietary fibers, beta-glucan, and other ingredients that may boost overall health and immunity. Effects of the isolated proteins, aqueous and ethanolic extracts of the Oyster on the production profiles and bio health markers in broiler birds were evaluated in the present research. Four
hundred and twenty, day-old Ross-308 broiler birds were distributed into seven groups like control (CONT) with no treatment and while six groups were orally supplemented with mushroom protein at a dose of 200 (Pr200) and 400mg/Kg BW (Pr400), an aqueous extract of mushroom at a dose of 200 (Aq200) and 400mg/Kg BW (Aq400) and ethanolic extract at a dose of 200 (Eth200) and 400mg/Kg BW (Eth400). There were sixty number of birds in each group. On day 28th of the experiment, 20 birds from each group was evaluated for cell-mediated immunity by assessing the lymphoproliferative reaction to previously injected Phytohemagglutinin-P (PHA-P; Toe web assay). The humoral immune reaction was evaluated in 20 birds of each group by antibody reaction to sheep red blood cells (sRBCs; Hemagglutinatin assay in serum). On day 42 (end of the trial); rest of the 20 birds from each group were slaughtered for blood collection. The serum was separated and evaluated for the oxidative stress, lipid profile, blood glucose level, and immune parameters. One-way analysis of variance (ANOVA), Tuckey range tests, and geometric mean titer (GMT) was used to statistically analyze the results. The Eth400 birds showed significantly (P<0.05) higher cell-mediated immune response in terms of lymphoproliferative response to PHA-P (1.1±0.04 mm) in comparison with the control group (0.09±0.18 mm). Humoral immune response in terms of immunoglobulins (total antibody) titer to sheep RBCs was also seen significantly higher in the Eth400 group (49.31±0.03 mm) as compared to CONT group (26.7±0.1 mm). Birds supplemented with mushroom extracts and protein revealed significantly greater growth performance, maximum in Eth400 (1.7±0.01). It has been observed that the mushroom extracts and isolated protein did reduce the oxidative stress in the broiler birds which was maximum in Eth400 group when compared to CONT. Conclusively, the oyster mushroom has health promoting effects in terms of production profile, as immunomodulator and antioxidant in broiler poultry.

Key Words: Pleurotus ostreatus, Broiler, Oxidative Stress, Production Profile, Immunomodulator

84 Egg yolk antibodies: A novel anti-diarrheal food ingredient. Jill W. Skrobarczyk1, Cameron L. Martin1, Mohammed Alabdali1, Sohini Bhattacharya, Suresh Pillai1,2, Luc R. Berghman1, 1Poultry Science, Texas A&M University, College Station, Texas, United States, 2National Center for Electron Beam Research, Texas A&M University, College Station, Texas, United States.

Rotavirus is the leading cause of severe gastroenteritis among infants and children. Infections are more prevalent in developing countries where access to health care and clean food and water is limited. Egg yolk antibodies (IgY) have a proven record of protection against enteric pathogens via oral passive immunization. They are able to withstand the harsh gut environment and are capable of reducing diarrhea in infected individuals. The aim of this study was to evaluate the protective efficacy of rotavirus specific egg yolk antibodies (IgY) generated against the Wa G1P[8] human rotavirus (HRV) strain. HRV was grown in MA104 cells to titers of 6.5 log TCID50. The virus was inactivated by electron beam irradiation at the lowest dose possible to maintain antigenicity. Virus particles were purified via ultracentrifugation using a cesium chloride gradient. White leghorn hens were immunized with the virus every 2 weeks until serum antibody titers peaked. Egg yolk antibodies were precipitated from eggs once the birds were hyper immunized and subject to ELISA and in vitro neutralization assays. High tittered (1:125,000) antibodies were obtained from the eggs after three immunizations. In vitro, the serum and anti-HRV IgY exhibited high neutralizing titers against the G1P[8] strain, 1:1700 and 1:500, respectively. Anti-HRV IgY generated against the irradiated virus demonstrated considerable recognition of the virus (ELISA) and neutralization in vitro. These data indicated the potential for a new cost effective prophylactic strategy to alleviate the global burden of rotavirus infections.

Key Words: rotavirus, egg yolk antibodies, IgY, passive immunization, prophylactic

85 A survey of gastrointestinal microbiota on commercial poultry farms in 4 countries in Latin America. Anne Ballou*, Andrés Vásquez, Kevin Garcia, Iluma Innovation Labs, Durham, North Carolina, United States, Biological Engineering, Universidad Nacional de Colombia, Medellín, Colombia.

The gut microbiome is the focus of intense study for its potential to impact animal health and performance, but the complex nature of its development, activity, and interaction with its host and surroundings limits the ability of researchers to interpret their results in this area. Studies on broilers in the US may not represent the host-microbe-environment relationship in broilers in India, or layers in Colombia. It is necessary, then, to explore the variation in the population, accounting for as many variables as possible. This baseline will allow producers and researchers to better understand the microbial population they are working with, its regulators, and what to expect when introducing new gut-health interventions. To begin building this baseline, we surveyed poultry microbial populations in Latin America across regions, farm sizes, and management strategies. Over 1500 samples were collected, representing 21 farms in Colombia, Ecuador, Guatemala, and El Salvador. Ileal, cecal, and fecal samples for 16S rDNA sequence analysis were collected from representative animals from each flock. Analysis was done using Qiime2, as well as R packages including Phyloseq, Vegan, DESeq2, and Metacoder. Preliminary results show that the microbiome is developed by 28 days of age, with similar levels of species richness in animals 28-42 days old. Interestingly, though animal age is a major factor in microbial composition, the differences in microbial populations between farms or regions appear to be greater than those due to age. Microbial populations tend to be
similar between animals at higher taxonomic levels, with Lactobacillus species dominating in the ileum and feces, and Ruminococcaceae, Lachnospiraceae, and Clostridial groups in the cecum. Farm-level differences come from changes in the taxa within these groups. For example, Lactobacillus salivarius is the dominant species in the ileum of birds in Ecuador, Guatemala, and El Salvador; while Colombian birds have a more mixed population of various Lactobacilli, as well as a larger component of unidentified bacteria. Understanding the implications of these differences could help producers select the most applicable gut health interventions for their needs and microbial populations. Similarly, broiler and laying hen microbiomes are comparable at the Family or Phylum level, but significantly different in their genera and species, raising questions about differences in the ways that these populations may affect the health and function of the animal. Current studies are sampling additional regions, and incorporating a broader range of nutritional, environmental and temporal variables, in order to better correlate microbial populations with desirable outcomes.

**Key Words:** Microbiome, Gut health, Bioinformatics

86 Characterizing the immune response of broilers to Campylobacter jejuni. Mohamad Mortada1,2, Douglas E. Cosby2, Ramesh Selvaraj1, 1Department of Poultry Science, The University of Georgia, Athens, Georgia, United States, 2Poultry Microbiological Safety and Processing Research Unit, USDA-ARS, Athens, Georgia, United States.

Campylobacter is one of the major foodborne pathogens that cause bacterial gastroenteritis worldwide. Handling and consumption of raw or undercooked poultry meat represent the highest risk of Campylobacteriosis in humans. The immune response responsible for the persistence of Campylobacter colonization is under-researched. Therefore, this study aimed to characterize the immune response of chickens to Campylobacter jejuni colonization through in vivo and ex vivo experiments. A total of 240 off-sex male broilers were weight-matched on d14 and randomly assigned to two treatments control and challenge. Birds in the challenge treatment were orally gavaged with 0.5 mL of 2.4 x 10^8 CFU/mL of Campylobacter jejuni (Strain A74C), while birds in the control treatment were mock-challenged with 0.5 mL of 0.85% saline. Campylobacter jejuni persisted in the ceca of broilers throughout the trial with bacterial colonization reached 4.9 log10 CFU/g on d35. Campylobacter also disseminated to the spleen (d21 to d28) and liver (d21) and was cleared by d35 from both internal organs. On d17, splenic mononuclear cells from challenged birds produced higher nitric oxide levels when compared to cells from the control birds, after ex vivo stimulation with LPS or lysed C. jejuni. Cecal tonsil MNCs from challenged birds were suppressed on d17 and d21, and this suppression was shown to be antigen-specific on d21. On d35, cecal tonsil MNCs from challenged birds had a significant increase in proliferation when stimulated ex vivo with the diluted lysed C. jejuni. Campylobacter jejuni significantly decreased IL-1β and TNF-α mRNA levels on d17, increased iNOS mRNA levels on d21, and increased IL-10 mRNA levels on d35 in the cecal tonsils (P < 0.05). In the spleen, C. jejuni challenge significantly increased IL-10, TGF-β, IL-1β, and TNF-α mRNA levels. In addition, challenge significantly increased TLR-4 mRNA levels on d28 in cecal tonsils and spleen. In conclusion, Campylobacter jejuni challenge initially suppressed the immunity in an antigen-specific response, upregulated both pro and anti-inflammatory cytokines in a balanced manner, and skewed, over time, the local immune response in the ceca towards an anti-inflammatory response seen by increased IL-10 mRNA levels and increased bacterial persistence until d35.

**Key Words:** Campylobacter jejuni, Mononuclear cells, T cell proliferation, Nitric Oxide, Cytokine gene expression

87 The development of immune system and function of broiler chicks in laminated cages. Bochen Song1,2, Dazhi Tang1, Hao Fan1,2, Liping Gan1, Shaojia Yan1, Guang Li1, Peng Li1, Yuming Guo1, 1College of Animal Science and Technology, China Agricultural University, Beijing, Beijing, China, 2Emory University, Atlanta, Georgia, United States.

The purpose of this study was to explore the developmental rules of the immune system and function of broiler chicks kept in laminated cages, and provide a scientific basis for the regulation of immune nutrition. A total of 106 male one-day-old Arbor Acres broiler chicks were selected, reared in laminated cages. They were sampled after euthanasia on day 1, 6, 13, 20, 27, and 34. Six chickens were selected on day 1, and twenty chickens were selected for sampling at other days of age. The concentrations of cytokines in serum samples were determined with commercially available chicken cytokine ELISA kits. The proportion of immune cells was analyzed through flow cytometry. A 3-((4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide assay was used to determine the peripheral blood lymphocyte proliferation response. The gene expression was assayed through RT-PCR. The experimental data was analyzed by One-way ANOVA of SPSS 20.0 software, and the measured indexes were compared with linear and quadratic curve polynomials. When P <0.05, the difference is significant. The results showed that broiler chicks had three development patterns of the immune system and function from day 1 to 34. The first pattern was Down-Up, and the lowest levels were basically during 6-13 days, including: specific cellular immunity items such as serum IL-1β, IL-4, IL-10, IFN-γ levels and splenic IL-6 mRNA level, humoral immunity items such as IgA level, mucosal immunity items such as ileal IL-2, IFN-γ / IL-4, Ilyz C and NF-kB mRNA levels. The second pattern was Up-Down, the highest levels were during 30-34 days, including: non-specific cellular immunity items such as peripheral blood mononuclear macrophage ratio, monocyte phagocytic activity, specific cellular immunity items such as peripheral
blood Th cell ratio, T cell and B cell proliferation activity, mucosal immunity items such as ileal CD4 receptor, TGF-β1 and IgA mRNA levels. The third pattern was Up-Up, including: non-specific cellular immunity items such as serum NO, C3 and C4 levels, specific cellular immunity items such as spleen index, peripheral blood IL-2, IFN-γ / IL-4, Tc cell ratio, splenic NF-kB mRNA level, specific humoral immunity items such as serum IgG level, mucosal immunity items such as ileal MHC-II, CD3d, TCRβ subunit, TCRζ subunit, IFN-γ and plgR mRNA levels, ileal mucosa sIgA level. It could be concluded that the immune system and its function not developed well during 6-13 days of age and the immune system didn’t mature until 30-34 days of age in broiler chicks kept in laminated cages. It is necessary to use additives in the period of 1-30 days of age to enhance the immune system function of broiler chicks.

**Key Words:** Immune development, Broiler, Immune function, Cytokine, Cage rearing

88 Modeling broiler industry loss to estimate the value potential of tracking the global burden of animal diseases. Kevin L. Watkins*1,2, Stephanie Rutten-Ramos1, Benjamin Huntington2, FoodFirst, LLC, Indianapolis, Indiana, United States, 2University of Liverpool, Liverpool, United Kingdom, 3Elanco Animal Health, Greenfield, Indiana, United States.

Disease prevalence and diagnostic data are critical for decision-making related to animal health, welfare, and productivity. While comprehensive disease and risk factor datasets are available for humans there is no equivalent for animals. The Global Burden of Animal Diseases (GBADs) project was initiated with the vision of standardizing animal disease classifications and incorporating public and private data into integrated datasets available for evidence-based economic analysis. In 2019, the GBADs team, led by the University of Liverpool and World Organisation for Animal Health (OIE) with funding from the Bill and Melinda Gates Foundation and the UK’s Department for International Development, conducted a global survey of 47 producers, processors, allied companies, and retailers along with 121 individuals from government and non-government agencies (Gov-NGO) involved in livestock and poultry systems. The survey showed that 74% of the private sector and 67% of Gov-NGOs already collect their own data related to disease prevalence. Over 90% of respondents said they would use a GBADs-like system if available. The top reasons for using GBADs were “for better disease prevention and treatment” and “faster response to disease outbreaks.” To estimate the value potential of GBADs, data from the National Chicken Council, FAOSTAT, broiler breed performance guides, and a subset of Elanco’s poultry health database (2008-2010) were used to model economic loss. Comparison between current production metrics and reported genetic potential of common broiler breeds estimates the potential to reduce live-production cost at more than USD 9 billion annually. Focusing on disease loss, necropsy data from over 1800 broiler complexes across 64 countries estimate that better diagnosis and earlier response to enteric disease could be worth USD 367 million annually. While GBADs would not completely eliminate enteric disease loss or the gap between genetic potential and current productivity, even modest improvements have significant value potential. Private- and public-sector survey and live-phase necropsy and production data suggest that a standardized system for disease and associated risk classification could improve and hasten decision-making related to the identification, prevention, and treatment of poultry diseases. Not only would a GBADs system improve animal health, welfare, and productivity directly, the availability of such data could also improve policies and prioritization of investments that advance One Health and achievement of the UN Sustainable Development Goals of health, environment, hunger, and poverty.

**Key Words:** broiler health, disease classification, economic analysis, disease loss

89 Candidate Gene for Chicken Alloantigen A. Robert L. Taylor*, Wioleta Drobik-Czwarno1, Janet Fulton1, Division of Animal and Nutritional Sciences, West Virginia University, Morgantown, West Virginia, United States, 2Hy-Line International, Dallas Center, Iowa, United States, 3Department of Animal Genetics and Conservation, Warsaw University of Life Sciences, Warsaw, Poland.

Chicken nucleated red blood cells have thirteen alloantigen systems (A, B, C, D, E, H, I, J, K, L, N, P, R) that have been identified via specific antibodies or molecular methods. Despite data showing that alloantigens influence economic traits, alloantigen B, the chicken MHC, is the sole system whose chromosomal location and gene products has been identified. This study sought to identify the chromosomal location and the product of the alloantigen system A gene. At least seven unique alleles occur in the A alloantigen system. Existing sequences of six tibred lines with previously determined A system genotypes were available. These lines were sequenced in pools on Illumina GAIIx platform using a paired-end protocol producing an average coverage of 15.8x across all samples. The Illumina sequence reads were aligned to chicken reference genome, build 5 (gallGal5.0) using the Burrows-Wheeler Aligner (BWA) v0.5.7 at the using default setting. GATK standard pipeline was used for variant calling. In addition, pools of DNA from A system genotyped individuals from outbred lines were produced and these were genotyped with the 600 K Affymetrix Axiom chicken SNP panel. Bioinformatic analysis for alloantigen A was conducted using both sequence data from inbred lines and the 600K SNP chip genotypes from outbred lines. For each SNP on 600k panel regression equation was developed where expected allele count (based on serology information) was used as response variable and signal intensity was used as explanatory variable. Coefficient of determination (R squared) generated based on regression analysis was used to select SNPs that segregated in accordance with expected pool genotypes and preselect regions for further analysis.
Salmonella Enteritidis population in cecal content at 1wk- and 2wk-post-challenge was decreased \( (P<0.05) \) by 8.26%, and by 11.18%, respectively, in birds immunized with CNP, compared to control. CNP vaccine did not alter \( (P>0.05) \) CD4+CD8+ T-cells percentages at 1wk- and 2wk-post challenge, when compared to control. CNP vaccine did not affect \( (P>0.05) \) IL-1β, IL-10 and iNOS mRNA amounts at 2wk-post-challenge, when compared to control. Findings demonstrate that the in-oovo administration of CNPs can induce a specific immune response against Salmonella and can decrease SE cecal colonization.

**Key Words:** Salmonella, Nanoparticles, Vaccination, In-oovo, Broiler

**92 Day of hatch exposure to Enterobacteriaceae and characterization of avian pathogenic E. coli on inflammation.** Kaylin Chasser\(^{*}\), Audrey F. Duff, Whitney Briggs, Kate McGovern, Johel Bielke, Lisa Bielke, Animal Sciences, Ohio State University, Columbus, Ohio, United States.

Early exposure to Enterobacteriaceae on day of hatch has been linked to gastrointestinal (GI) inflammation, which can have important impacts on overall health. Exposure to Salmonella enterica serovars and Escherichia coli (EC) at the hatchery influence intestinal integrity and inflammation. An experiment was conducted to test wild-type Enterobacteriaceae poultry intestinal isolates for impacts on growth performance and inflammation. Treatment included a non-inoculated control (NC), an oral dose of \( 10^3 \) CFU/chick of EC LG (LG), EC Huff (Huff), Salmonella Enteritidis E2 (E2), or Salmonella Typhimurium (ST) at \( 10^4 \) CFU/chick on DOH. Serum was collected for alpha-1-acid glycoprotein (A1GP) and fluorescein isothiocyanate dextran (FITC-d) analysis on d2, 8, 15, 22, and 29. Yolk sacs were collected, weighed, and plated for bacterial enumeration on d8 and 15. All data were subject to analysis of variance with means separated using Tukey’s HSD. No differences were observed for YS weight, YS retention, YS bacterial enumeration, or FITC-d. However, an interaction was observed between treatment and time for A1GP, with a significant effect observed on every day except d29 \( (p<0.05) \). While A1GP concentrations of E2 remained relatively steady from d2 to d8, NC, LG, and Huff all displayed a sharp decline in circulating A1GP of over 400μg/mL, while ST resulted in a sharp increase, from 914.42μg/mL to 1766.70μg/mL. Both Huff and LG mirrored this sharp increase between d8 and 15, and between d22 and 29, respectively. PCR analysis was completed on LG and Huff to test for the presence of avian pathogenic EC (APEC) genes for classification as APEC strains. Of the six APEC genes tested, hlyF, Iron, ituA, iss, ompT, and sitA, the latter three were present in both EC strains tested, resulting in classification as APEC strains. This may contribute to the effects observed in EC treatments, where increases in A1GP, though not at the same time, may be a result of the colonized APEC strain expressing more virulence genes due to an opportune environment within the GI tract. In conjunction with the influence pioneer colonizing bacteria have on the intestinal tract, early exposure to pathogens have the potential to affect inflammation and may result in greater susceptibility to opportunistic pathogens. By understanding how early exposure to Enterobacteriaceae alters patterns of inflammation over time, in addition to monitoring for APEC strains and their influence on intestinal health, treatments and prophylactic measures can be targeted to counteract these effects.

**Key Words:** Enterobacteriaceae, E. coli, Salmonella, gastrointestinal inflammation, alpha-1-acid glycoprotein

**93 Enhancement of host defense peptide gene expression and disease resistance by butyrate, forskolin, and lactose in broiler chickens.** Qing Yang\(^{*}\), Melanie Whitmore, Sydney Stewart, Kelsy Robinson, Glenn Zhang, Animal and Food Sciences, Oklahoma State University, Stillwater, Oklahoma, United States.

Modulating the synthesis of endogenous host defense peptides (HDP) has emerged as a promising antimicrobial alternative approach to disease control and prevention. This study is aimed at exploring three natural HDP-inducing compounds, namely, butyrate, forskolin (FSK), and lactose, to mitigate two economically significant poultry diseases. We examined a possible synergy among three natural products in HDP gene induction in chicken HD11 macrophages and jejunal explants. HD11 cells or jejunal segments were treated with butyrate, FSK, lactose individually or in combination for 24 h, which was followed by RNA isolation and gene expression analysis. One-way ANOVA and Tukey post-hoc test were applied for data analysis. The three compounds synergized to boost the expression of β-defensin 3 (AvBD3), AvBD8, AvBD9, and AvBD10 genes in HD11 cells \( (P < 0.05) \). Moreover, the expression of claudin-1, claudin-5, tight junction protein-1, and mucin-2 genes were synergistically improved by butyrate, FSK, and lactose \( (P < 0.05) \). These compounds also showed a synergy in promoting AvBD9 and AvBD10 transcription in jejunal explants \( (P < 0.05) \). We further evaluated the efficacy of these compounds on disease resistance in chicken models of necrotic enteritis and coccidiosis. Day-of-hatch Cobb male broiler chicks were randomly allocated to one of three treatments which included an uninfected control, an infected control, and an infected group supplemented with sodium butyrate (1 g/kg diet), FSK (10 mg/kg), and lactose (10 g/kg). Necrotic enteritis was induced through the challenge of 10^6 oocysts of Eimeria maxima on d 11 followed by Clostridium perfringens (10^5 CFU) through d 14 to 16. Lesion scoring and intestinal digesta collection were performed on d 18. Results showed that the medication of three compounds reduced mortality \( (P < 0.0001) \), counteracted growth retardation \( (P < 0.05) \), alleviated jejunal lesions \( (P < 0.01) \), and suppressed jejunal colonization of C. perfringens \( (P < 0.01) \) as compared with the infected control. Chicks were inoculated with 2×10^8 oocysts of E. maxima on d 10 in the
coccidial trial. The supplementation of compounds prevented weight loss (P < 0.05), mitigated intestinal pathology of coccidiosis (P < 0.0001), and reduced fecal oocyst shedding (P < 0.01) as compared with the infected control birds at 7 days post-infection. Collectively, our results suggested that butyrate, FSK, and lactose compounds have the potential to be further developed as novel antimicrobial alternatives for the control and prevention of necrotic enteritis, coccidiosis, and possibly other infectious diseases in poultry.

**Key Words:** antibiotic alternatives, host defense peptide, necrotic enteritis, coccidiosis, chicken

**94 Effect of supplementation of a protected complex of biofactors and antioxidants in broiler chickens undergoing early life stress.** Cristiano Bortoluzzi¹, Ludovic Lahaye², Krystel Marcil², Catherine Chaput³, Michael H. Kogut¹, Elizabeth Santin², ¹Poultry Science, Texas A&M Agrilife Research, College Station, Texas, United States, ²Jefo Nutrition Inc., Saint-Hyacinthe, Quebec, Canada, ³USDA-ARS, Southern Plains Agricultural Research Center, College Station, Texas, United States.

We hypothesized that the supplementation of different antioxidant molecules through feed would alleviate the negative effects of early life stress in broiler chickens. Therefore, we evaluated the dietary supplementation of a protected complex of biofactors and antioxidants P(BF+AOx) on the growth performance, immune parameters, and antioxidant activity of broilers submitted to early life stressors induced by the combination of a double Infection Bronchitis (IB) vaccination followed by a cold stress. The experiment consisted of two treatments: feed supplemented or not with P(BF+AOx) (Jefo Nutrition Inc., Saint-Hyacinthe, Qc, Canada) distributed to an amount of 720 one-day old male Ross 308 chickens placed in pens of 30 birds (12 replicates per treatment). Birds were double vaccinated against IB (MILDVAC-Ma5TM) by spray at the hatchery and submitted, on d 3, to an acute cold stress with a temperature drop from 32°C to 20-23°C during 48 h. Feed intake (FI), Body weight gain (BWG), and feed conversion ratio (FCR) were calculated weekly. On d 7 and 15, samples of lung were collected for expression of immune-related genes analysis, and serum was collected to evaluate the antioxidant status. All the data were analyzed by ANOVA using the software SAS (SAS 9.4). There was a trend towards improved FCR on d 7 (P = 0.10) and BWG on d 14 (P = 0.06) in birds fed P(BF+AOx) supplemented diets. From d 1 to 21 and d 1 to 28, it was observed that the dietary supplementation of P(BF+AOx) significantly improved BWG (P < 0.05) by 3.6 and 3.8%, respectively, and FCR (P < 0.05) by 1.2 and 1.8%, respectively. Considering the overall experimental period (d 1 to 35), the dietary supplementation significantly enhanced BWG (P = 0.03) by 4% and tended to improve FCR (P = 0.07) by 2.3%. There was no difference between treatments on d 7 and d 15 for TBARS and glutathione peroxidase activity. The glutathione reductase (GR) activity, on d 15, was significantly higher in birds fed diets supplemented with P(BF+AOx) compared to the control diet fed birds (P = 0.04). It was observed that the dietary supplementation downregulated (P = 0.02) the expression of IL-1β on d 7 but did not change IL-10 nor INF-γ expression. The dietary treatment did not change the expression of the genes on d 15. Based on the current results, it can be concluded that the supplementation of this protected complex of biofactors and antioxidants improved growth performance, increased the activity of the antioxidant enzyme GR, and modulated the expression of IL-1β in the lungs of chickens undergoing an early life stress.

**Key Words:** antioxidants, broilers, immune system, infectious bronchitis, early life stress

**95 Ulvans activates avian monocytes and neutrophils.** Nathalie Guriec², Frédéric Bussy¹, Maria Garcia³, Maria Angeles Rodriguez⁴, Benoît Quéro⁵, Matthieu Le Goff⁶, Pi Nyvall-Collen⁷, ¹Olimx Group, Brehan, France, ²Brest University & Brest Medical School, Brest, France, ³R&D Breizh, Pluméliau, France.

Recent research has highlighted the potential of in-feed marine polysaccharides (ulvans) from the cell wall of green macroalgae as a reliable strategy to reinforce animal’s immune response. The effect of an extract from Ulva armoricana (MSP®IMMUNITY) on birds’ monocytes and heterophils was evaluated in vivo. Three hundred male broilers (Ross 308) of 28 days of age were randomly distributed in 4 groups of 25 birds each and were given ulvan per os at different concentrations (0, 10, 25, 50 mg/l) at day 0 of the trial (28 days of age). Blood samples (1 milliliter per animal) were daily collected from day 0 to day 3. Nitric oxide (NO) concentration and β-D-glucuronidase activity were measured individually in the plasma, the latter via the 4-methyl-belliferone formation. A dose- and time-dependent NO release was observed with maximal concentrations at day 1 with values of 9.99 ± 0.85 μM for the negative control, 29.72 ± 1.34 μM with 10 mg/l ulvan, 48.22 ± 1.51 μM with 25 mg/l ulvan, and 81.8 ± 6.50 μM with 50 mg/l ulvan. The groups without ulvan supplementation did not present any statistically significant variations over time, with NO concentrations between 10.41 ± 0.71 μM and 9.34 ± 0.35 μM. Increased ulvan concentrations led to higher 4-methyl-belliferone synthesis. A peak of β-D-glucuronidase activity at day 1, swiftly declining, with values no longer statistically different from that of the negative control at day 3. At day 1, β-D-glucuronidase in the plasma allowed 4-methyl-belliferone concentrations to rise to 6.29 ± 0.48 μM for the negative control, 48.7 ± 2.85 Mm with 10 mg/l ulvan, 74.67 ± 3.38 μM with 25 mg/l ulvan, 89.61+/4.23 μM with 50 mg/l ulvan. In the groups without any ulvan added, no variation was observed with 4-methyl-belliferone concentrations ranging from 6.12 ± 0.55 μM to 6.63 ± 0.39 μM. In vivo, when given per os, the ulvan extract modulates the activity,
directly and/or indirectly of key players of the chicken innate immune system, i.e., heterophils and monocytes. This innovative bioactive molecule can thus play an important role within the strengthening of the immune response.

**Key Words:** Algae, Immunity, Monocytes, Neutrophils, Polysaccharides

96 **The use of a direct ELISA to identify Blackhead resistant turkeys.** Christina S. Sigmon*, Alessandro Ferrarini, Robert Beckstead, Prestage Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, United States.

Blackhead disease is caused by a protozoan parasite, *Histomonas meleagridis*. Infected turkeys display clinical signs of drooping head, depression and sulfur-colored droppings. The ceca become inflamed and the liver becomes necrotic, leading to death. In the field, mortality can range from a few birds to the whole flock. It is unknown if this variation is due to bird genetics, management or a combination thereof. To determine if genetic resistance is apparent in commercial turkey flocks, two trials were conducted with flocks 1 (males) from parental line A and flock 2 (females) from parental line B with each flock containing 2,000 poults. All birds were neck tagged for identification. On day 18, each bird was inoculated with 50,000 *Histomonas* cells given in the cloaca. Flocks received 2-3 subsequent infections until the trials were terminated on day 80. Mortality and lesion scores were collected throughout the study. Blood was collected for ELISA assays at trial termination. The survivor percentage was calculated based on the population of poults alive at 80 days from the initial flock. To detect if survivors had an immune response, a *H. meleagridis* direct sandwich ELISA was used with a response considered if the sample surpassed the calculated cut off value of the average of the controls minus the standard deviation of the controls times 3. Flock 1 (males) had 11.2% survivors with 5.1% having no lesions. Of the 52 plasma samples tested from flock 1 birds showing no clinical signs, all were positive for an immune response. Flock 2 (females) had 9.8% survivors and 3.7% with no lesions. Of the 76 plasma samples tested from flock 2 birds showing no clinical signs, 61 had an immune response, with 15 below the cut off threshold. As a control 11 plasma samples for birds that survived, but had signs of Blackhead disease, were tested. 10 out of 11 sensitive samples showed an immune response, indicating that not all turkeys showing clinical signs of Blackhead disease initiate an antibody response that can be detected. It is possible that the ELISA-negative birds may still have been infected by *H. meleagridis* and recovered, but did not mount an antibody-inducing response. The identification of turkeys that have an antibody response to *H. meleagridis*, but no disease signs, supports the hypothesis that a subset of turkeys is resistant to Blackhead disease. However, this data also suggests that future research should analyze turkeys that are resistant to Blackhead disease, but do not initiate an antibody response.

**Key Words:** Histomonas, ELISA, turkeys, immune, resistant

97 **In ovo gamma-tocopherol vitamin E reduced the incidence of deep pectoral myopathy (green muscle disease) in broilers.** Lizza Macalintal*, Anthony Pescatore1, Michael Ford1, Merlin Lindemann1, Tuoying Ae2, *University of Kentucky, Lexington, Kentucky, United States, ’Alltech, Inc., Nicholasville, Kentucky, United States.

The current study evaluated the influence of γ-tocopherol vitamin E (GTVE) on hatchability, post-hatch performance, and incidence of deep pectoral myopathy (green muscle disease) using *in ovo* application. Green muscle disease (GMD) is a localized degenerative condition of the *pectoralis minor* (p. minor). The inability of the *p. minor* to expand as a result of exertional myopathy can lead to ischemic, necrotic, and atrophied muscle. Affected *p. minor* progress from pinkish hemorrhagic appearance to gray-greenish discoloration resulting in downgrades during post-harvest. For the *in ovo* study, hatching eggs from Ross 308 breeder hens were allocated randomly to 6 treatment groups consisting of 50 eggs/group. Eggs were hand-injected with 100ul of canola oil or 30 IU GTVE (mixed tocopherols 95, DSM) in canola oil on ED (embryo day) 12 or 18.5. Control eggs included non-injected and no treatment eggs. Fertile eggs received injections through the yolk sac at ED 12 or via the amnion at ED 18.5. At post-hatch through d 49, all chicks were fed with a corn-soybean meal basal diet. At d 35 and d 42, three birds/pen were subjected to simulated wing-flapping (SWF). Each broiler received 6-8 cycles of repeated raising and lowering to approximately 1.9m/cycle. For each replicate pen, three birds were subjected to SWF, whereas another set of three birds without SWF served as the control. Results indicated that injecting fertile eggs with canola or GTVE at ED 12 reduced the hatchability (P<0.05) of fertile eggs compared to the control groups. At ED 18.5, hatchability did not differ among the treatment groups (P>0.05). Total hatchability was lower for ED 12 vs. ED 18.5 (P<0.05). Growth performance and feed efficiency through d 49, was not affected by *in ovo* injection of canola or GTVE in comparison to the control. At 14 d post-initial SWF (d 49), *p. minor* of euthanized SWF and non-SWF broilers were inspected visually and recorded for GMD lesions. After episodes of prolonged wing flapping, GMD was correlated directly with the SWF exercises. *P. minor* of control birds not subjected to SWF were without lesions. In SWF birds, susceptibility to GMD was lower for the birds that received *in ovo* GTVE regardless of the time of ED injections; control>canola>*in ovo* (P<0.05). *In ovo* GTVE reduced the incidence of GMD in SWF birds (45.82%) compared to 62.5 and 91.58% for canola and control groups. In conclusion, *in ovo* administration of γ-tocopherol vitamin E through the yolk sac at ED 12 or via the amnion at ED 18.5 reduced the incidence of green muscle disease in broilers. Further research with γ-tocopherol vitamin E
should be conducted with other muscle myopathies that occur in broilers.

**Key Words:** in ovo, vitamin E, green muscle disease, wing-flapping, deep pectoral myopathy

98 Not Presented.
Nevertheless, more complete vaccination program and stocking densities, drinking and feeding spaces were met. Outdoor access were mainly used and requirements for table eggs (45.5%), fowls for exhibitions (36.4%) and meat production allowance was not reached. Farmers supplied Chantecler (AQVC). Attempts to fully supply egg or meat Québec (RPQ) and Association Québécoise de la Volaille email was sent to individuals listed in the membership. The survey was conducted during fall 2019 by ITA. An agreement signed in 2009 with marketing boards allow each of 10 producers to breed 100 chickens m-2) where they continued growing till 49 d of age. Feed conversion ratio of 18.0 kg of feed per kg of weight at hatch, and at different ages, in industrial poultry. There exists evidence of the influence of egg weight on live weight at hatch, and at different ages, in industrial poultry. However, that information is not available for local poultry genetic resources such as Creole chickens of Mexico. The objective of this study was to characterize egg weight, live weight, and relationships among those traits in Creole chickens of Mexico. Two hundred and forty-three straight run Creole chicks, belonging to an experimental randombred flock, were studied from 0 to 49 d of age. Birds were individually identified and raised in battery brooders from 0 to 42 d of age and then transferred to floor (10 chickens m-2) where they continued growing till 49 d of age. Chickens were fed a diet with 20.5% CP and 2,900 kcal ME kg-1. Water and feed were provided ad libitum during all the length of the study. The studied variables were: weight of the egg from which each bird was hatched (EW); and live weight of the chicken at 0, 7, 14, 21, 28, 35, 42, and 49 d of age (LW0, LW7, LW14, LW21, LW28, LW35, LW42, and LW49, respectively). Data were analyzed by MEANS and CORR procedures of SAS. EW varied from 32.0 to 72.0 g with and mean weight of 52.6±0.5 g. Live weight at different ages varied as follow: LW0 (17.0-49.5 g), LW7 (26.5-69.0 g), LW14 (42.5-134.0 g), LW21 (75.0-224 g), LW28 (98.5-341.0 g), LW35 (129.0-510 g), LW42 (267.0-580.5 g), and LW49 (328.0-780.0 g). Average values of LW0, LW7, LW14, LW21, LW28, LW35, LW42, and LW49 were estimated as 34.1±0.4, 48.7±0.6, 85.8±1.3, 143.8±2.2, 220.0±3.1, 312.3±4.5, 407.2±9.6, and 541.4±12.7 g, respectively. It was found that EW is highly correlated with LW0 (r = 0.79, P < 0.0001). Although the correlation of EW with other live weights was also significant (P < 0.0001), they were of lower magnitude: LW7 (r = 0.42), LW14 (r = 0.35), LW21 (r = 0.41), LW28 (r = 0.34), and LW35 (r = 0.37). Finally, the estimated LW0:EW ratio was 0.650±0.004. In conclusion, there is a great variation in terms of egg weight and live weight of Creole chickens of Mexico. Hatching weight of the chick is highly associated to weight of the egg from which the bird hatched; but this relationship does not continue as the bird gets older.

Key Words: egg weight, live weight, Creole chickens, Mexico

Management and Production

99  A survey on sustainable development of the Chantecler chicken breed. Jean-Marc Larivière*, Institut de technologie agroalimentaire, La Pocatière, Québec, Canada.

Celebrating in 2021 its Centennial year of approval by the American Poultry Association’s Standard of Perfection, Chantecler is a dual-purpose chicken breed. First interest for the breed led Trappist monk Brother Wilfrid to present results at the Xth World Poultry Congress in Ottawa, Canada, in 1920. The breed exists in white and partridge feather colour morphs but black and buff are currently being elaborated. Whole-genome resequencing recently identified cold-resistant related genes and adaptation to climatic conditions. Its small cushion comb and wattles with plumage make it a unique cold hardy chicken with distinct genetic originality. An agreement signed in 2009 with marketing boards allow each of 10 producers to breed 100 hens with 15 males to produce 30,000 hatching eggs per year, supply table eggs from 500 laying hens and around 42,000 kg from 20000 chicks per year. However, ten years later, very little has been achieved to save Chantecler from extinction. The aim of this survey is to describe breeding, egg-laying and growing management practices in Québec. The survey was conducted during fall 2019 by ITA. An email was sent to individuals listed in the membership directory and provided by Les Racines Patrimoniales du Québec (RPQ) and Association Québécoise de la Volaille Chantecler (AQVC). Attempts to fully supply egg or meat production allowance was not reached. Farmers supplied table eggs (45,5%), fowls for exhibitions (36,4%) and meat (13,6%). Building with windows and free range with outdoor access were mainly used and requirements for stocking densities, drinking and feeding spaces were met. Nevertheless, more complete vaccination program and biosecurity measures could be applied with a priority to establish a pathogen-free nucleus. Average fertility and hatching rate varied from 50-95% and at different ages varied as follow: LW0 (17.0-49.5 g), LW7 (26.5-69.0 g), LW14 (42.5-134.0 g), LW21 (75.0-224 g), LW28 (98.5-341.0 g), LW35 (129.0-510 g), LW42 (267.0-580.5 g), and LW49 (328.0-780.0 g). Average values of LW0, LW7, LW14, LW21, LW28, LW35, LW42, and LW49 were estimated as 34.1±0.4, 48.7±0.6, 85.8±1.3, 143.8±2.2, 220.0±3.1, 312.3±4.5, 407.2±9.6, and 541.4±12.7 g, respectively. It was found that EW is highly associated to weight of the egg from which the bird was hatched; but this relationship does not continue as the bird gets older.

Key Words: Chantecler, chicken, sustainability, survey, breed

100  Relationship among egg weight and live weight traits in Creole chickens of Mexico. José G. Reyes-Bello1, Oliver J. Juárez-Beltrán1, Leonardo Osio-Orihuela1, Osvaldo Fernández-Ipíña2, Diego Zárate-Contreras2, Arturo Pro-Martínez2, Juan M. Cuca-García2, Leodán T. Rodríguez-Ortega3, Fernando González-Cerón1, 1Department of Animal Science, Chapingo Autonomous University, Texcoco, State of Mexico, Mexico, 2Livestock Program, College of Postgraduates Campus Montecillo, Texcoco, State of Mexico, Mexico, 3Polytechnic University of Francisco I. Madero, Francisco I. Madero, Hidalgo, Mexico.

There exists evidence of the influence of egg weight on live weight at hatch, and at different ages, in industrial poultry. However, that information is not available for local poultry genetic resources such as Creole chickens of Mexico. The objective of this study was to characterize egg weight, live weight, and relationships among those traits in Creole chickens of Mexico. Two hundred and forty-three straight run Creole chicks, belonging to an experimental randombred flock, were studied from 0 to 49 d of age. Birds were individually identified and raised in battery brooders from 0 to 42 d of age and then transferred to floor (10 chickens m-2) where they continued growing till 49 d of age. Chickens were fed a diet with 20.5% CP and 2,900 kcal ME kg-1. Water and feed were provided ad libitum during all the length of the study. The studied variables were: weight of the egg from which each bird was hatched (EW); and live weight of the chicken at 0, 7, 14, 21, 28, 35, 42, and 49 d of age (LW0, LW7, LW14, LW21, LW28, LW35, LW42, and LW49, respectively). Data were analyzed by MEANS and CORR procedures of SAS. EW varied from 32.0 to 72.0 g with and mean weight of 52.6±0.5 g. Live weight at different ages varied as follow: LW0 (17.0-49.5 g), LW7 (26.5-69.0 g), LW14 (42.5-134.0 g), LW21 (75.0-224 g), LW28 (98.5-341.0 g), LW35 (129.0-510 g), LW42 (267.0-580.5 g), and LW49 (328.0-780.0 g). Average values of LW0, LW7, LW14, LW21, LW28, LW35, LW42, and LW49 were estimated as 34.1±0.4, 48.7±0.6, 85.8±1.3, 143.8±2.2, 220.0±3.1, 312.3±4.5, 407.2±9.6, and 541.4±12.7 g, respectively. It was found that EW is highly correlated with LW0 (r = 0.79, P < 0.0001). Although the correlation of EW with other live weights was also significant (P < 0.0001), they were of lower magnitude: LW7 (r = 0.42), LW14 (r = 0.35), LW21 (r = 0.41), LW28 (r = 0.34), and LW35 (r = 0.37). Finally, the estimated LW0:EW ratio was 0.650±0.004. In conclusion, there is a great variation in terms of egg weight and live weight of Creole chickens of Mexico. Hatching weight of the chick is highly associated to weight of the egg from which the bird hatched; but this relationship does not continue as the bird gets older.

Key Words: egg weight, live weight, Creole chickens, Mexico

101  The effect of housing environment on commercial...

Consumer demand for eggs from alternative housing systems is driving the layer industry towards greater use of extensive housing systems. However, limited research is available on how these systems affect egg production characteristics of commercial white egg layers. Four housing systems were evaluated under typical industry conditions, including standard cages (C), enrichable colony cages (CC), enriched colony cages (E) and cage-free (CF). We hypothesized that hens in cages would perform more favorably than hens in alternative systems. Seven different white egg laying strains were housed in the different housing systems and managed according to standard husbandry practices and stocking densities. Data collection for the strains began at 17 weeks of age, with a base period of 28 days for feed weigh-backs and egg quality measurements. The housing system had a significant ($P<0.0001$) effect on all egg production characteristics measured, including feed consumption (g/bird/day), egg production rates (% hen-day and % hen-housed), feed efficiency (g egg/g feed), mortality rate (%) and egg weights (g/egg). Research has shown that white egg layers preform the best in caged environment in all ways. We observed that the white egg layers had optimum egg production and feed conversion in cages, however the white egg layers also had the lowest mortality rate and highest feed efficiency in cage-free environments. Hen-day egg production of these birds was $81.2\pm 0.3\%$ for CC, $83.4\pm 0.4\%$ for CF, $84.2\pm 0.3\%$ for E and $84.6\pm 0.2\%$ for C. Hen-housed production was the lowest for the CC system at $70.0\pm 0.3$ followed by E at $79.1\pm 0.3$, CF at $79.2\pm 0.5$ and finally C at $80.2\pm 0.3$. Feed consumption was the lowest for the cage-free birds at $101.8\pm 0.5$ followed by C at $105.4\pm 0.3$, E at $107.4\pm 0.3$ and CC at $111.4\pm 0.3$. Feed efficiency was the poorest in the CC system at $0.450\pm 0.001$ followed by E at $0.481\pm 0.0018$, C at $0.498\pm 0.0016$ and finally CF at $0.500\pm 0.0026$ g egg/g feed. The cage-free birds had the lowest mortality rate with an average period mortality of $5.22\pm 2.82\%$ followed by C at $15.3\pm 2.69\%$, E at $15.6\pm 3.03\%$, and finally CC at $31.3\pm 3.03\%$. Finally, the caged hens laid the heaviest eggs at $61.6\pm 0.08$g followed by CC at $61.2\pm 0.09$g, E at $60.9\pm 0.09$g and CF at $60.5\pm 0.13$g. White egg layers exhibited significantly better production characteristics in caged housing systems, however they also showed similar feed efficiency and lowest mortality rate in cage-free environments.

Key Words: White Egg Layers, Housing Systems, Egg Production, Feed Efficiency, Mortality Rate


The present study was undertaken to investigate the impact of heat stress on nutrient digestibility and tibia and reproductive traits, and changes in laying performance, egg qualities, fecal volatile fatty acids and serum parameters in laying hens. Hens were raised in three temperature-controlled facilities with constant humidity, either normal temperature (LT; 22°C) or heat stress considered being moderate (MT; 27°C) or severe (HT; 32°C) for 42 days. Relative humidity was set at 50% in all facilities. Experimental design of this research is completely randomized design. Two adjacent cages were considered an experimental unit. All data were analyzed using the general linear model procedure of SAS. Feed intake were consistently low ($P<0.001$) in HT hens compared with those in LT or MT over the period of 42 days. Egg production kept markedly ($P<0.05$) or numerically ($P>0.05$) low in hens exposed to HT vs. LT or MT. Egg mass and egg weight were consistently low ($P<0.01$) in hens exposed to HT compared with those raised under LT or MT. On the other hand, feed conversion ratio and frequency of dirty and cracked eggs were not significantly affected ($P>0.05$) during the experimental period. HT-treated hens consistently had low ($P<0.05$) eggshell thickness and strength, eggshell weight compared with NT- or MT-treated hens. HT hens had lower ($P<0.01$) relative oviduct weight and less number of large yellow follicles compared with those raised under LT or MT conditions at 42 days. Tibia traits measured at 42 days were not affected by any of heat treatments. Fecal volatile fatty acids tended to be higher in HT-exposed laying hens throughout the experiment. It was noted that digestibilities of neutral detergent fiber and dry matter exhibited lowest ($P=0.001$) in hens exposed to HT vs. LT or MT environments. Our study indicates that heat stress consistently lowers feed intake, egg production and eggshell qualities during the experiment and dramatically impairs fiber digestibility in laying hens.

Key Words: heat stress, laying performance, fiber digestibility, volatile fatty acid, laying hens

103 The effect of housing environment and hen age on white shell egg solids and quality. Kenneth E. Anderson*, Benjamin N. Alig, Peter R. Ferket, Ramon Malheiro, Prestige Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, United States.

The egg industry is shifting production systems from cage confinement to cage-free based on consumer preferences and focus groups. Consequently, demand for cage-free eggs is increasing and the layer industry is rapidly moving towards greater use of cage-free housing systems. However, limited research is available on how these systems affect egg quality characteristics and solids of eggs produced from white egg layers in these systems. The objective was to evaluate the quality of eggs from white laying hen strains based in four housing systems standard cages (C), enrichable colony cages (CC), enriched colony cages (E) and cage-free (CF). We hypothesized that hens in these housing systems would produce eggs with similar quality
characteristics. Seven different white egg-laying strains were housed in the four housing systems and managed according to standard husbandry practices and stocking densities. In selected periods, all eggs were evaluated for a set of physical characteristics including egg weight, Haugh unit, yolk color (TSS, York, England), shell strength, and vitelline membrane strength (Texture Analyzer, Surry, England). Statistical analysis was done using JMP 14 and significant means differences between period and housing system were determined using Tukey’s HSD. The differences observed among the three periods were highly significant (P<0.01) for all egg characteristics evaluated, which are expected as hens age. Eggs were lighter from hens housed in the CF system than the other systems (P<0.01), while the Haugh unit scores (HU) were higher (P<0.01). The higher HU was substantiated by the elevated USDA Grade A % and lower % of Extra Lg eggs. Eggs from hens housed in the CF had higher shell breaking strength (P<0.01), requiring more force to break (5,001g), followed by C (4,200g), while E (4,259g) and CC (4,105g) showed a similar shell strength, respectively. The vitelline membrane strength was not affected by the housing system. The egg solids % increased (P<0.01) in eggs from the CF system (24.76%) over the eggs from the E system (24.13%), while both the C and CC were intermediate at 24.54 and 24.39%, respectively. In conclusion, the housing systems of CF appears to improve the external and internal egg quality and solids content, which may be due to the better interaction of the layer hens with some beneficial variables from the environment.

Key Words: white egg layers, housing systems, egg quality, egg solids

The effect of housing environment on egg quality and solids of commercial brown egg layers. Benjamin N. Alig*#, Peter R. Ferket, Ramon Malheiro, Kenneth E. Anderson, Prestige Department of Poultry Science, North Carolina State University, Wake Forest, North Carolina, United States.

Consumer demand for eggs from more extensive housing systems is driving a shift in the layer industry towards these housing systems. However, there is little research that explores the effects of different housing environments on the egg quality and solids from brown egg-laying chickens. A correlation between housing environment and egg quality has been shown, but this effect has not been studied with enriched and enrichable cages under typical modern US conditions. The objective of this study was to identify a correlation between housing environments and egg quality and egg dry matter weight (egg solids) Five housing systems were evaluated in this study including caged (C), enrichable (EN), enriched (E), cage-free (CF) and free-range (R). Three brown egg laying strains were housed in the different housing systems and managed according to standard husbandry practices and stocking densities. Data collection for the strains began at 17 weeks of age, with a base period of 28 days for feed weighbacks and egg quality measurements. Egg quality measurements included egg weight (g), percent grade As, Bs and loss (%) and egg solids (%) (dry egg matter percentage). Statistical measurements were taken using JMP 14 and data was analyzed using least squares method. Significant differences between treatments were determined using Tukey’s HSD. Housing system had a highly significant (P<0.05) effect on egg weight (g/egg) as well as percent grade A, percent grade B, and loss, however there was no significant housing effect on egg solids percent. The birds in the E environment laid the lightest eggs at 60.5±0.1g followed by, E and EN hens at 61.1±0.2g, CF hens at 61.3±0.2g and R at 63.9±0.2g. The E environment resulted in the lowest yield of grade As at 87.8±0.4%, followed by EN at 87.8±0.4%, C at 89.2±0.4%, CF at 96.3±0.6%, and R at 947.6±0.6%. CF hens had the highest grade B proportions at 1.41±0.2% followed by, E at 1.16±0.1%, EN at 1.16±0.1%, E at 0.78±0.1% and R at 0.68±0.2%. Conversely, the E environment had the highest percent loss at 11.0±0.4%, followed by EN at 10.5±0.4%, C at 9.0±0.4%, CF at 2.3±0.5% and R at 1.7±0.5%. This data shows that the birds in a range environment produced the largest eggs and the highest percent grade As and egg solids was not affected by housing environment.

Key Words: Brown Egg Layers, Housing Systems, Egg Quality, USDA Sizes, USDA Grades

Prevalence of avian pathogenic Escherichia coli in cage-free W-36 pullets reared under monochromatic blue and normal LED lights. Ishab Poudel*, Nikhil Nuthalapati, Anuraj Sukumaran, Li Zhang, Mary Beck, Aaron Kies, Pratima Adhikari, Poultry Science, Mississippi State University, Starkville, Mississippi, United States.

Avian pathogenic Escherichia coli (APEC) is a major avian pathogen that causes economic losses to the layer industry worldwide. In this 16-wk study, we evaluated the prevalence of E. coli with APEC associated genes in samples collected from cage-free Hy-Line W-36 pullets, reared under normal light-emitting diode (LED) and monochromatic blue LED lights. Five hundred, day-old Hy-Line W-36 chicks were randomly placed cage-free in two rooms, one with normal LED and the other with blue-LED lights. The light treatment was applied to the monochromatic blue LED room for 24 hours a day and the normal LED room as per Hy-Line Management Guide. Within each room, litter and fecal samples from four different quadrants, and cloacal and tracheal swabs from four random birds were collected at the 4th, 8th, 12th, and 16th weeks of the trial. Single colonies were isolated on MacConkey agar and confirmed for E. coli using real-time PCR by detecting the presence of the ybbw gene. Positive samples were further screened for the presence of APEC associated virulent genes, iroN, ompT, hlyF, iss, and iutA. A chi-squared test was performed to compare the difference in the presence of APEC associated virulent genes between the two treatments and sample type. The results showed a
One of the most important blood-feeding ectoparasites of Riverside, California, United States is *Ornithonyssus sylviarum*. Among all samples that were confirmed to be *E. coli*, 461 (46.42%) were found to have one or more APEC associated genes, with a prevalence of 80.37, 78.97, 79.90, 86.91, and 98.59% for *iroN, ompT, hlyF, iss,* and *iutA,* respectively. Further, the prevalence of one or more virulent genes in colonies isolated from the litter, fecal, cloacal swabs and tracheal swabs samples was 39.84, 41.41, 33.33 and 53.23%, respectively (*P* = 0.013). Our results conclude that regardless of the light treatment, the highest prevalence of APEC associated genes was found in colonies isolated from the trachea. Further study is needed to understand the mechanism of APEC infection through the respiratory tract and its spread into other internal organs, which will help us to formulate effective managemental strategies to reduce their infection in laying hen.

**Key Words:** Avian Pathogenic *E. coli*, cage-free, laying hen, monochromatic LED

### 106 A northern fowl mite infestation impacts cage-free layer performance and welfare between 17 and 49 weeks of age

Rachel Jarrett, Darrin M. Karcher, Marisa Erasmus, Deana R. Jones, Kailynn Scoles, Amy Murillo, Animal Sciences, Purdue University, West Lafayette, Indiana, United States, US National Poultry Research Center, Egg Safety and Quality Research Unit, USDA Agricultural Research Service, Athens, Georgia, United States, Entomology, University of California Riverside, Riverside, California, United States.

One of the most important blood-feeding ectoparasites of poultry, the northern fowl mite (*Ornithonyssus sylviarum*), has the potential to negatively impact production and welfare in a poultry flock. A northern fowl mite (NFM) infestation can elicit a host immune response that leads to decreased egg production, anemia, irritation to flocks and personnel, profit loss, and death to hens in extreme cases. The objective of this study was to examine the effects of an NFM infestation on cage-free laying hen performance and welfare quality between 17 and 49 weeks of age. At the Purdue University Poultry Unit, 800 Tetra Brown hens (n=200 per room) were housed in four cage-free rooms using the Big Dutchman Colony 2+ System. Two rooms served as the control group and two rooms were infested with NFM. In the two NFM rooms, infestation took place at 24 weeks of age on 2% of the initial hen population (n=4 hens per room, or n=8 hens total) and a second infestation to boost the NFM population took place on 2% of the initial hen population. Mite checks were conducted periodically on 25% (50 hens) in all rooms between weeks 25 and 38 to track initial spread of infestation. Monthly mite counts were taken on all hens beginning at 28 weeks. All hens were assessed monthly using the Welfare Quality® Assessment Protocol for Poultry to determine NFM impact on hen welfare. Egg production and mortality were recorded daily and case weights were recorded weekly. Data were analyzed in SAS® using the GLM procedure and an ANCOVA and all significant statistical differences were reported at *P* < 0.05. Proportion of hens with a mite infestation was treated as the covariate. Percent livability followed the Tetra Brown management guide expectation (9% and 8% lower for control and NFM, respectively). Treatment had an effect on eggs per hen housed, mortality, feather damage on the head and neck, and skin lesions (*P* < 0.05), with the control group outperforming the NFM group for all. The proportion of hens infested with mites had an effect on feather damage on the back, crop, head, and neck (*P* < 0.05). Overall, the northern fowl mite (NFM) infestation negatively affected egg production, mortality, feather coverage, and resulted in an increase in skin lesions, and the proportion of hens infested with mites had a negative effect on feather coverage in a cage-free setting. Proper measures to prevent and control a northern fowl mite infestation in a cage-free setting should be taken in order to prevent a negative impact on egg production and hen welfare.

**Key Words:** Cage-free, Layer, Welfare, Poultry, Ornithonyssus sylviarum

### 107 Quantifying sex and line differences in growth parameters in turkeys

Sasha van der Klein, Owen Willems, Martin J. Zuidhof, R&D, Hendrix Genetics, Kitchener, Ontario, Canada, University of Alberta, Edmonton, Alberta, Canada.

Body weight (BW) is one of the main metrics in meat-type animal breeding programs. Yet, few publications are available on the BW – age relationship in turkeys. The objective of this study was to create an integrative BW model, from which sex and line effects could be derived. It was hypothesized that compared with females, the model would predict increased BW at the same age and a later age at inflection (age which BW gain occurred at the highest rate) for males and male line turkeys. BW data was provided by Hendrix Genetics (Kitchener, ON, Canada). All contemporary groups of birds were brooded in the same facilities for 6 weeks, after which they were transferred to grow out barns. Birds were raised on wood shavings litter and ad libitum fed a standard commercial turkey diet. Water was provided ad libitum. BW data were collected from randomly sampled individuals in consecutive flocks (contemporary groups of birds with the same hatch date) over a period of 129 weeks and averaged for each flock per age, line, and sex. Data included a total of 913 observations from hatch to 20 weeks of age of a male and a female line of Large White turkeys of both sexes. R was used to fit a modified Gompertz model using the nls function and the nlstools package. The model included a fixed effect of sex and line on all estimated coefficients: \[ W_t = \]
The objective of this study was to compare estimated parameters and the adjusted coefficient of determination \( (R^2_A) \), of the Gompertz growth model, when it is adjusted using data collected of Creole chickens of Mexico at different time intervals (d). Eighty-eight male chicks from a randombred experimental flock, were reared from 0 (hatching) to 133 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. Individual live weight (LW) (g) was recorded every third day, and also every week, from 0 to 63 d of age. Later, LW was recorded weekly until 133 d of age. Four LW databases with different recording interval (RID) were established to estimate parameters of the Gompertz model. In order to achieve this, LW data from some days were not taken into account, these data were considered missing. The recording intervals were: RID1, every third day (0-63 d of age) and every fourteen days (63-133 d of age); RID2, every third day (0-63 d of age) and every seven days (63-133 d of age); RID3, every seven days (0-133 d of age); RID4, every seven days (0-63 d of age) and every fourteen days (63-133 d of age). Hatching weight \( (W_0) \), asymptotic weight \( (W_A) \), age of maximum growth \( (t) \), initial growth rate \( (L) \) and rate of decay \( (K) \) were the estimated parameters for Gompertz model. \( R^2_A \) was the selection criterion to identify the best RID. The number of observations in each database was: RID1, \( n= 2376 \); RID2, \( n= 2816 \); RID3, \( n= 1760 \) and RID4, \( n= 1320 \). Data were analyzed using the NLIN procedure of SAS (Marquardt algorithm). Estimated parameter values were as follows: \( W_0 \) (g), RID1: 37.3, RID2: 36.9, RID3: 36.6 and RID4: 37.1; \( W_A \) (g), RID1: 2985.9, RID2: 2972.6, RID3: 2965.1 and RID4: 2977.6; \( L \) (g/d), RID1: 0.0953, RID2: 0.0959, RID3: 0.0964 and RID4: 0.0957; \( K \) (g/d), RID1: 0.0217, RID2: 0.0218, RID3: 0.0219 and RID4: 0.0218. The \( R^2_A \) values were RID1: 0.9466 vs RID2: 0.9412 vs RID3: 0.9265 vs RID4: 0.9410. In conclusion, data collected according to RID1 gave a better fit to describe the growth curve of Creole chickens of Mexico using Gompertz model. However, the other studied recording intervals also have an optimal fitting (\( R^2_A \) 0.90).

**Key Words:** modeling, gain, weight, production

**108 Frequency of data collection on the estimation of parameters of the Gompertz model to describe the growth of Creole chickens of Mexico.** Diego Zárate-Contreras1, Fernando González-Cerón2, Juan M. Cuca-García1, Arturo Pro-Martínez3, Gustavo Ramírez-Valverde4, Omar Hernández-Mendoza1, Jaime Gallegos-Sánchez2, Josafhat Salinas- Ruiz1, Rosalia Ordez-Contreras1, Miguel Á. Matus-Aragón1, Artemio J. Vargas-Galicia1, 1Livestock Program, College of Postgraduates Campus Montecillo, Texcoco, State of Mexico, Mexico, 2Department of Animal Science, Chapingo Autonomous University, Texcoco, State of Mexico, Mexico, 3College of Postgraduates Campus Cordoba, Cordoba, Veracruz, Mexico.

The objective of this study was to compare estimated parameters and the adjusted coefficient of determination \( (R^2_A) \), of the Gompertz growth model, when it is adjusted using data collected of Creole chickens of Mexico at different time intervals (d). Eighty-eight male chicks from a randombred experimental flock, were reared from 0 (hatching) to 133 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. Individual live weight (LW) (g) was recorded every third day, and also every week, from 0 to 63 d of age. Later, LW was recorded weekly until 133 d of age. Four LW databases with different recording interval (RID) were established to estimate parameters of the Gompertz model. In order to achieve this, LW data from some days were not taken into account, these data were considered missing. The recording intervals were: RID1, every third day (0-63 d of age) and every fourteen days (63-133 d of age); RID2, every third day (0-63 d of age) and every seven days (63-133 d of age); RID3, every seven days (0-133 d of age); RID4, every seven days (0-63 d of age) and every fourteen days (63-133 d of age). Hatching weight \( (W_0) \), asymptotic weight \( (W_A) \), age of maximum growth \( (t) \), initial growth rate \( (L) \) and rate of decay \( (K) \) were the estimated parameters for Gompertz model. \( R^2_A \) was the selection criterion to identify the best RID. The number of observations in each database was: RID1, \( n= 2376 \); RID2, \( n= 2816 \); RID3, \( n= 1760 \) and RID4, \( n= 1320 \). Data were analyzed using the NLIN procedure of SAS (Marquardt algorithm). Estimated parameter values were as follows: \( W_0 \) (g), RID1: 37.3, RID2: 36.9, RID3: 36.6 and RID4: 37.1; \( W_A \) (g), RID1: 2985.9, RID2: 2972.6, RID3: 2965.1 and RID4: 2977.6; \( L \) (g/d), RID1: 0.0953, RID2: 0.0959, RID3: 0.0964 and RID4: 0.0957; \( K \) (g/d), RID1: 0.0217, RID2: 0.0218, RID3: 0.0219 and RID4: 0.0218. The \( R^2_A \) values were RID1: 0.9466 vs RID2: 0.9412 vs RID3: 0.9265 vs RID4: 0.9410. In conclusion, data collected according to RID1 gave a better fit to describe the growth curve of Creole chickens of Mexico using Gompertz model. However, the other studied recording intervals also have an optimal fitting (\( R^2_A \) 0.90).

**Key Words:** modeling, gain, weight, production

**109 Effects of maternal growth pattern on broiler chicken performance and carcass traits.** Mohammad Afrouziyeh1, Nicole Zukiwsky, Martin J. Zuidhof, University of Alberta, Edmonton, Alberta, Canada.

The current study investigated the effect of accelerated maternal growth on offspring performance and carcass traits. We hypothesized that advancing broiler breeder pubertal phase growth and relaxing the amount of growth restriction during pubertal phase would increase offspring BW and gut weight. In a randomized controlled trial, a total of 40 female broiler breeders were randomly assigned to 10 growth trajectories that were implemented using a precision feeding system. The maternal growth trajectories were designed using a 3-phase Gompertz model with the form: \( BW = \sum_{i=1}^{3} W_{mi} \exp[-b_i(t-t_{inf})^i] \) where \( W_{mi} \) was the amount of BW gain that accrues in the phase of interest (kg); \( b \) was the rate of maturing; \( t \) was age (wk); \( t_{inf} \) was the inflection point (wk); \( i \) was the growth phase (1 = 1 to 3). The maternal growth curves were designed with 2 levels of BW gain in phase 1 (discrete variable). \( W_{mi} \) was the amount of BW gain in the first phase (pre-pubertal) which was estimated by fitting the breeder-recommended BW target to a 3-phase growth model (CON), or 10% higher (HIGH). The second phase (pubertal) inflection point \( (t_{inf}) \) was advanced by 0, 5, 10, 15, or 20% (continuous variable) in both CON and HIGH treatments. Settable eggs were collected from hens at 35 and 42 wk of age and incubated. On average, 12 chicks per maternal treatment from each maternal age were raised to 35 d of age and fed using a precision feeding system. Analysis of covariance was conducted on all dependent
variables using the MIXED procedure of SAS, with maternal age and $W_m$ as sources of variation and $t_{m2}$ as covariate. Maternal $W_m$ affected progeny cumulative gain where the heaviest offspring were resulted from the HIGH maternal treatment ($P = 0.05$). Chick BW increased by advancing $t_{m2}$ ($P = 0.013$). Chick BW increased by 0.038 and 0.036 g per each day that the maternal pubertal growth phase was advanced in the CON and HIGH treatments, respectively. The effect of advancing $t_{m2}$ on offspring gut weight depended on maternal age ($P = 0.035$) and on FCR, fat pad, pectoralis major and minor muscles depended on $W_m$ ($P = 0.05, 0.024, 0.016$ and 0.014, respectively). Liver weight increased by maternal age and $W_m$ ($P = 0.05$). Consistent with hypotheses, increased maternal pre-pubertal target BW and accelerating maternal pubertal growth rate increased progeny performance by increasing their BW gain and digestive tract development. In conclusion, relaxing the level of maternal growth restriction before and during pubertal phase increased broilers’ hatch BW, performance and some carcass parts weight.

**Key Words:** broiler breeder, carcass, feed restriction, maternal effect, multi-phasic growth

**110 Comparison of a traditional female diet to a hen diet with organic zinc on rooster semen quality and reproductive performance.** Kelly M. Sweeney*, Drew Benson, Carla Aranibar, Monique Franca, Benton Hudson, Duarte Neves, Santiago Garcia-Gomora, Jeanna Wilson, Poultry Science, University of Georgia, Athens, Georgia, 1Population Health, University of Georgia, Athens, Georgia, 2Aviagen Inc., Huntsville, Alabama, United States, 3Zinpro Corp., Eden Prairie, Minnesota, United States, 4Department of Medicina y Zootecnia de Aves, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico City, Mexico.

In the US, male broiler breeders 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 2750 kcal/kg energy, 14% crude protein, and 3% total calcium was mixed (Aviagen, 2016) and split into two diets providing either 110 ppm zinc from zinc sulfate (Hen-ZnS) exclusively or a 50:50 combination of organic zinc and zinc sulfate (Hen-OZn) in the mineral premix. All treatment groups were fed equal feed amounts from 25 to 65 wks of age. In this study, a basal diet with 2750 kcal/kg energy, 14% crude protein, and 3% total calcium was mixed (Aviagen, 2016) and split into two diets providing either 110 ppm zinc from zinc sulfate (Hen-ZnS) exclusively or a 50:50 combination of organic zinc and zinc sulfate (Hen-OZn) in the mineral premix. All treatment groups were fed equal feed amounts from 25 to 65 wks of age. A total of 63 Ross Yield Plus roosters were assigned to each dietary treatment (9 replicates, 7 roosters per replicate) starting at 25 wks of age. 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 4 wks from 33 to 65 wks. Individually caged Ross 708 hens were artificial inseminated with 0.05 mL pooled and diluted semen from a replicate group of 7 males. Semen was diluted with Avian Buffer to 7.5 x 10^7 sperm. Eggs were collected for 14 days’ post-insemination and incubated. An objective of this study focused on whether the organic Zn supplemented diet would improve rooster semen production and quality, as well as the morphological structure and integrity of the kidneys and testes. Data were analyzed by SAS 9.4 SLICE and means separated by LSD. Significance level was P<0.05. Neither diet had a significant influence on rooster body weight, semen volume, concentration, or mobility to 65 wks of age. Percentage of roosters producing semen was not influenced by mineral supplementation to 65 wks. The dietary supplementation also had no significant impact on fertility. All roosters were euthanized at 65 wks of age, and rooster body weight and testes weight measured for calculation of relative testes weight. There were no significant differences between treatments for testes or body weight, or relative testes weight. Histological observations were not significantly different in the morphological structure of the testes or kidneys. While specific diet formulations for adult roosters may be warranted to enhance reproductive characteristics, the alteration from Zn sulfate alone to a mix of Zn sulfate and organic Zn had limited impact on reproductive measurements in this trial.

**Key Words:** Organic Zinc, Rooster Semen Quality, Fertility, Testes Morphology, Kidney Morphology

**111 Effect of heat treatment during storage on initial stages of duck embryo development.** Serdar Özlü*, Carlos Eduardo Bites Romanini, Roger Banwell, Okan Elibol, Department of Animal Science, Faculty of Agriculture, Ankara University, Ankara, Turkey, 2Petersime NV, Belgium.

The aim of the study was to examine the effect of the heat treatment during storage on blastoderm development of duck hatching eggs. The eggs were obtained from a Pekin duck breeder flock at 36 wk of age and stored overnight at 17°C and 75% RH in farm storage room. A total of 1728 eggs were transferred in a climate controlled vehicle to the hatchery storage room (17°C and 75% RH) within 2h. The eggs were randomly assigned to 2 groups of heat treatment, each operating at eggshell temperature of either 95°F (35.0°C) or 100°F (37.8°C) in a Petersime ReStore™ machine for two successive days. To assess blastoderm development during heat treatment, a randomly subset of 30 embryos was determined at every 3h intervals (initial-0, 3, 6, 9, and 12h). Embryos were isolated from the yolk by using the filter ring technique and staged according to Eyal-Giladi and Kochav (EGK) and Hamburger and Hamilton (HH) reference tables. The development of blastoderm data were subjected to one-way-ANOVA using the Minitab 14.0. The significant differences among the treatment duration means were determined by Duncan’s multiple range test at 0.05 significance level. The initial blastoderm stage before the heat treatment was on average EGK8.8 on both days. The developmental stage of blastoderm ranged between EGKVI and HH3+ after 12h of heat treatment. The average blastoderm development of the treatments were EGK9.4,
between the two nest box designs (raised: mean = 64.9% +/- 34.4; floor: mean = 63.17% +/- 34.7; p = 0.1552). The mating behavior, including mating attempts and successful copulations, was more frequent in pens with floor nest boxes compared to pens with nest boxes on raised slats (p = 0.04). The litter area in pens with floor nest boxes was slightly larger which could explain the higher frequencies of mating behavior and floor eggs. Furthermore, our birds were rather young and therefore their mobility might not yet have been impaired. An important aspect of the egg laying behavior is finding a secluded and undisturbed place. As males tend to avoid the slatted area, the hens may have experienced less disturbances in the nest boxes on the raised slats. These results suggest that raised slats may be beneficial for the welfare of broiler breeder hens as less mating behavior was occurring, while accessing the nest boxes on the raised slats did not seem to be impaired.

Key Words: Broiler Breeder, nest box choice, animal welfare

113 Hatchability and embryo mortality of birds provided red, white, or blue LED light during incubation. Janessa Henry*, Bruce Rathgeber, Nancy McLean, Xujie Li, Janice MacIsaac, Dalhousie University, Truro, Nova Scotia, Canada.

It is no surprise that hatcheries want to maximize their production, with optimal hatchability, minimal embryo mortality and late hatchers. Historically most incubation units are not equipped with lights, which could be of value to the developing embryo. The objective of this study was to determine if the use of LED lighting during incubation of multiple strains of chickens affected hatchability and embryo mortality. Two replicate trials were performed using the following strains: Ross 308, Cobb 500, Lohmann LSL Lite and a 1978 random bred broiler line from the University of Alberta. Eight incubators were used, setting 50 eggs per strain per trial. Incubation lighting consisted of 4 treatments: dark (no light), white LED, dim to red LED, and dim to blue LED. Lighting treatments had a 12L:12D photoperiod and were replicated for each trial. Chicks were pulled from the incubators at 521 h and counted to calculate hatchability. All remaining unhatched eggs were opened to classify stage of embryo development. Categories were infertile, early, mid and late dead and chicks found live after 521 h. This experiment was a completely randomized block design, blocked by trial and incubation room (group of 4 incubators). Data were analyzed in SAS 9.4 using the GLIMMIX procedure with a p-value < 0.05 as the level for alpha. No differences were found between light treatments for hatchability or any of the embryo mortality categories. However, the p-value for the number of chicks found live after 521h approached significance (P=0.06), indicating there may be reason to re-evaluate the impact of incubation in the dark (10.3%) compared to incubation with red light (2.7%). Significant differences were found between strains for hatchability, mid and late dead and chicks hatching late. Strain differences were expected as the genetic background
differs greatly between the older genetics and current day broilers and layers. This study can conclude that lighting was not found to be detrimental to hatchability or embryo mortality in the strains of chickens studied.

Key Words: Hatchability, embryo mortality, light, broiler, layer

114 Effects of reduced incubation temperature starting at 12 days of incubation on post-hatch broiler performance. Katie E. Elliott*, Ethan Dehart, Saman Fatemi, Abdul Mohssen Alqhtani, Ayoub Mousstaaid, Kayla Bannister, Wei Zhai, David Peebles, Poultry Science, Mississippi State University, Starkville, Mississippi, United States.

The objective of the current study was to assess the effect of a lowered incubation temperature starting on d 12 of incubation on broiler production (BW, FCR, processing yields, and woody breast score) through 6 wk of age. Ross 708 broiler hatching eggs were incubated in 4 incubators at a set temperature of 37.5°C until d 12 of incubation. At d 12 of incubation, 2 of the incubators were adjusted to a set temperature treatment of 36.7°C (cool) and the other 2 remained at 37.5°C (standard) until hatch completion. The chicks were removed from their incubators upon completion of hatch for each treatment. The chicks were feather sexed and sex-separately reared in floor pens in a randomized complete block design with an initial 16 birds per pen across 32 pens. Each incubation temperature treatment and sex combination were represented once in each of 8 blocks. Bird and feed weights were recorded weekly on a pen-basis. The data were analyzed for the main and interactive effects of incubation temperature and sex. No difference in hatch success was found between treatments (P=0.2279); however, hatch delay was deferred 22 hours by the cool treatment. The FCR was lower for the cool treatment during the first 7 d post-hatch (0.0107); however, after d 7 no further FCR differences were found (all P>0.2). Mean BW at placement did not differ due to incubation temperature (P=0.9150); however, starting at 7 d (P=0.0010) and weekly through 6 wk post-hatch, the cool treatment birds had a significantly lower BW as compared to the standard treatment birds. By 6 wk of age, the standard treatment birds averaged 83g heavier than the cool treatment (P=0.0190). Males were significantly heavier than females on wk 2 (P=0.0295), wk 5 (P<0.0001), and wk 6 (P<0.0001). Mortality occurred at a rate of 2.3% for both the standard males and the cool female treatments and at 0.8% for both the cool males and the standard female treatments. By processing at 6 wk of age, whole carcass weights were heavier for the standard treatment birds (P=0.0203) and for males (P=0.0001). The severity of woody breast was not affected by incubation temperature treatment (P=0.1813), although it was greater for males than females (P=0.0068). The results of this study show the lasting effects of a lowered incubation temperature through processing age in broilers. A diminished incubation temperature starting at 12 d of incubation to a set point of 36.7°C does not appear to offer any production benefit.

Key Words: incubation, temperature, woody breast, broiler, processing

115 The impacts of graded levels of stocking density on the performance, health, and welfare of turkey hens to 11 weeks of age. Sameeha Jhetam*, Kailyn Beulac, Karen Schwean-Lardner, Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Stocking density (SD) affects economic return for turkey production and impacts the health and welfare of birds. Most studies focus on toms rather than hens, thus a gap exists in the literature. This study (two trials) evaluated the impacts of SD on turkey hen performance, health and welfare to 11 wk of age. Nicholas Select hens (n=3550 pouls/trial) were randomly placed in one of four final estimated SD treatments of 30, 40, 50, or 60 kg/m² in open rooms (67.5 m²) with four replications per treatment (trt). Feeder and drinker space were equalized on a per bird basis and air quality (CO₂ and ammonia) was tested: ventilation was adjusted to equalize air quality across all trt. Mortality and culls were recorded daily and necropsied for cause of death. At wk 8 and 11, footpad, gait, feather condition, and cleanliness scores, and flock uniformity were evaluated (30 birds/replicate). Incidences of aggressive pecking were recorded daily, and birds were treated with a deterrent or culled depending on severity. 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 11, BW decreased linearly as SD increased (8.36, 8.35, 8.30, 8.19 kg; P=0.05). Feed consumption decreased linearly as SD increased during wk 8-11 (P<0.01) and decreased quadratically as SD increased over the 0-11 wk period (15.19, 15.29, 15.08, 14.90 kg; P=0.04). SD had no impact on BW gain, feed-to-gain ratio, percent mortality, or uniformity. Average footpad scores worsened at wk 8 as SD increased (linear; 1.43, 1.55, 1.86, 1.80; P=0.03), but no relationship was noted at wk 11. Average feather condition scores (sum of five body parts) were poorer in the high SD trt at wk 8 (linear; 17.96, 18.06, 17.30, 16.78; P<0.01) and 11 (linear; 17.09, 16.01, 15.68, 14.63; P<0.01). Average feather cleanliness scores were poorer in the high SD trt at wk 8 (linear; 1.69, 1.78, 2.64, 3.15; P<0.01) and wk 11 (linear; 1.79, 2.37, 2.80, 3.00; P<0.01). Incidence of aggressive pecking plus culls for aggressive damage (%) demonstrated a linear increase as SD decreased (7.88, 7.60, 4.05, 2.67%; P=0.01). Average gait scores were not affected by SD trt. The results indicate that high SD impacts production (final BW and feed consumption) and negatively impacts bird health and welfare due to poor footpad, feather condition and...
cleanliness scores. However, low SD negatively affects bird welfare due to increased incidence of aggression.

**Key Words:** body weight, footpad lesions, aggressive pecking, feather condition, feather cleanliness

**116 Dietary additives, coccidiosis and Fenbendazole treatment alter fecal moisture to various degrees in turkey poults.** Elle Chadwick*, Robert Beckstead, Prestage Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, United States.

Treatment-induced gut irritation is commonly used in experimental settings to mimic increased fecal moisture and wet litter observed in turkey production. However, the extent of gut irritation from different treatments is not always known. Therefore, a model was created in order to determine the expected fecal moisture based on different treatments provided to turkey poults. Fifty-six poults were placed in wire battery cages on day of hatch (eight poults per cage). Poults were provided feed and water ad libitum and given a turkey starter diet in mash form that was formulated with high Betaine (Diet 1). On day 17, the following treatments were provided: Diet 1, a diet with an imbalanced inclusions of crude protein, amino acids, salt, and phosphorus (Imbalanced), Diet 1 with 2 lbs/ton of magnesium sulfate (MgSO₄), Diet 1 with half of the corn replaced with coarse corn (Coarse Corn), Diet 1 with fat replaced with rancid fat (Rancid Fat), Diet 1 with a 10X dosage of a commercial coccidia vaccine (Cocci), and Diet 1 with poults given an oral inoculation of Fenbendazole (0.05 mL/kg bird) for five days (Wormer). Fecal samples were collected on days 17 (prior to treatments), 23 and 25 with three replicates per treatment per day. Samples were dried in an oven set at 55°C for 48 hours and fecal moisture was calculated based of feces and dry matter weight. Treatments and days were compared in JMP Pro 14 using GLM and means were separated using Tukey HSD with significance considered at P ≤ 0.05. On day 17, all cages had similar fecal moisture except for Rancid Fat, which was excluded for day 17 analysis due a water leak. On days 23 and 25, MgSO₄ and Cocci had the greatest fecal moisture content and Diet 1 had the least with significant differences found between treatments. The Imbalanced, Rancid Fat, Coarse Corn and Wormer did not differ significantly on day 23. The Imbalanced, Coarse Corn and Wormer did not differ significantly on day 25. Within each treatment, an age effect was seen where older birds had significantly less fecal moisture. The only exemption was the Cocci treatment, where poults were suffering from coccidiosis and had the least amount of fecal moisture on day 23 with an increase on day 25. From this data, it can be inferred that fecal moisture will vary based on age of the poult and can be manipulated to various extremes using different treatments.

**Key Words:** fecal moisture, turkey, coccidiosis, fenbendazole, feed additives

**117 Comparing litter to partially slotted flooring materials on tom turkey performance and litter moisture.** Mariah M. Huberty*, Jeanine A. Brannon*, Kevin Janni*, Carol Cardona*, Sally L. Noll*, Department of Animal Science, University of Minnesota, Saint Paul, Minnesota, United States, Department of Bioproducts and Biosystems Engineering, University of Minnesota, Saint Paul, Minnesota, United States, Department of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, Minnesota, United States.

Partially slotted flooring (PSF) has been adopted for broiler breeders in which a section of the floor is covered with slats. Interest for use in turkey rearing stems from the potential to alleviate excessive litter moisture. This study evaluated the use of PSF in comparison to a bedded system. The first hypothesis was that live performance traits such as BW, feed consumption, average daily gain, breast defects (button and blister), gait and feather condition scores would be comparable between slotted floor (SF) materials and conventional systems spare footpad scores and litter moisture. The second was that processing traits including footpad scores, breast defects, and condemnations would be comparable between SF materials and conventional systems. Treatments included an all pine wood shaving litter control (T1) and two SF materials; Double L Classic Red Rooster flooring (T2) and Tenderfoot Calf Mesh (T3). Six-hundred male turkeys (Large White, Nicholas) were assigned to pens (50/pen) at 6 wk of age across a randomized block design with 4 replicates per treatment. Performance (BW, feed intake), footpad scores (0-2, Berg, 1998), feather condition score (0-3, Welfare Quality Consortium, 2009), gait score (0-5, Kestin, 1992), and litter moisture (LM) was assessed through 18 wk of age. At a commercial processing plant, data was collected for footpad score; carcasses were evaluated for breast defects, and parts trim. Data were averaged by pen and ANOVA conducted to determine the treatment significance (Statistix v.10) (P<.05). Mean differences were identified through Tukey testing. A lowered ADG of 271 g for T2 was noted during 15-18 wk of age as compared to T1 and T3 with gains of 282 and 281 respectively (P<.05). Higher feather score (dirtier plumage) was observed for T1 (1.93) vs 1.13 and 1.33, for T2 and T3, respectively, at 18 wk (P < .03). Remaining performance characteristics were not different. Litter moisture increased with age, at 15 wk (P<.07) and at 17 wk (P<.05) between T1 and PSF. At 17 wk, LM for T1, T2 and T3 was 42, 34 and 38%, respectively. For processing traits, whole leg trim was 2.4, 0.0 and 0.6% trim, respectively (P<.007) for T1, T2, and T3. Breast buttons were more prevalent (P<.03) in PSF, 0.5% for the control vs 6.4 and 6.6%, respectively. Breast blisters averaged 22.8, 33.7 and 43.9% for T1, T2, and T3, respectively (P<.03). Partially slotted floor system lowered LM which may have in turn improved feather cleanliness and reduced processing leg trim. However, breast skin defects were more prevalent regardless of flooring material.

**Key Words:** Turkey, litter moisture, partially slotted flooring, carcass quality, live performance
Mitigating disease and reducing potential for cross-contamination in commercial flocks using robotics. Colin Usher*, Aerospace Transportation Advanced Systems Lab Food Processing Technology Division, Georgia Tech Research Institute, Atlanta, Georgia, United States.

A virus is spreading across the globe and a highly pathogenic avian influenza was confirmed in a commercial poultry flock recently. This has dealt a double blow to the poultry industry and highlights the need for safe and effective bio-safety practices, for both humans and birds. Researchers have spent several years developing a robot that can tend to poultry flocks. A primary motivating factor is to reduce the chance of cross-contamination and introduction of disease into the flocks. The idea is a simple one: the more that a robot can do, the less a person needs to intervene, reducing their need to enter the houses and therefore reducing the opportunity for contamination. There are robot systems available now for commercial flocks. Each carry out a single task such as monitoring, encouraging movement, and disinfecting. However, humans still have to enter the house daily to carry out manual tasks. The research team set out to design a robot that can carry out as many tasks as possible, reducing and even eliminating the need for a human to enter the house at all. The team developed a robot using a commercial-off-the-shelf chassis from Super-Droid-Robots. The robot uses a RP-lidar low-cost laser scanner, an Intel RealSense 2D and 3D sensor, a small end-effector from uArm capable of picking eggs, and a custom developed arm for removing mortality. All testing was performed in close partnership with the University of Georgia’s Poultry Science Department, testing in small-scale houses stocked with chickens having the same density of commercial houses. A set of behaviors allowing the robot to navigate autonomously was developed. Quite simply, chickens mostly move out of the way, allowing it to move freely. It must also be able to identify eggs. Artificial intelligence routines utilizing real data was collected in the field and used as input for training neural networks. A robot arm was added to the platform and routines for approaching and automatically removing floor eggs was developed. Finally, the robot must be able to remove mortality. A prototype arm capable of picking up chickens was fabricated. The robot has operated fully autonomously for over 200 hours. Egg picking capabilities of the robot were field tested showing a success rate of 90%. The prototype arm for mortality removal was tested, successfully lifting a 20 pound weight. The robot discussed in this document is one tool that has the potential to reduce contamination and disease in commercial flocks simply by reducing the requirement for humans to enter the facilities. While quantitative research needs to be conducted comparing disease incidents with flocks managed by humans versus those managed by robots, there is promise.

Key Words: Robotics, management, Autonomous, mitigation, reduction

Bedding and stocking density influenced the relationship of litter characteristics to footpad dermatitis in market turkey hens. Gabriella Furo¹,²*
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Stocking density (SD) and bedding material (B) impacts bedding condition which can influence footpad dermatitis, an important welfare indicator which is associated with high litter moisture. Alternative bedding materials, such as Giant miscanthus grass (MG) may be suitable and affordable bedding material for poultry production, because of fluctuation in bedding price and accessibility of pine wood shavings (WS). The objective of this study was to investigate the effect of SD and B on litter characteristics and footpad dermatitis. Day-old turkey hens (N=1096, Hybrid Converter) were assigned to six treatments (2 x 3 factorial, 4 pens/treatment) and studied for 14 weeks in a randomized block design. The two bedding materials were MG and WS and three SD (4.3 hens/m² (LD), 5.4 hens/m² (MD), and 7.1 hens/m² (HD)). Miscanthus was chopped into 5 cm lengths and all pens bedded to 10 cm depth. At 6 and 14 wk of age, litter samples were taken and footpads of every turkey were evaluated (0- no lesion and 2- severe, Berg, 1998) in each pen. Litter was analyzed for moisture; and, particle size distribution using a series of 10 sieves with openings ranging from 4.76 mm to 0.15 mm. Data were subjected to ANOVA with significance at P < 0.05. Spearman correlation ascertained the relationship among moisture, particle size distribution and footpad score. Footpad dermatitis results were reported earlier (Furo et al., 2017). At 6 wk, litter moisture was not different among study factors. At 14 wk, litter moisture increased as SD increased (48%, 51% and 58%, for LD, MD and HD, respectively), and litter moisture was higher on MG than WS (55.7% vs. 49%). The MG had larger particle size with most of the MG bedding retained by the largest sieve opening compared to WS at 6 wk (52 vs. 26%) and 14 wk (62 vs. 50%). Density did not influence the particle size distribution at 6 or 14 wk of age (P > 0.05). Bedding with larger particle size correlated with higher litter moisture at 6 and 14 wk (r=0.55 and 0.67, respectively) and higher average footpad score at 6 and 14 wk (r=0.81 and 0.67, respectively). In conclusion, more severe footpad score was associated with MG bedding, greater litter moisture, HD, and large particle size. Bedding material with smaller particle size should be studied in the future. Project support by USDA NIFA no. 2016-67015-24457.
Key Words: Giant miscanthus grass, bedding, turkey, stocking density, footpad dermatitis

120 Examination of poultry litter as a potential source for airborne pathogens. Amrit Pal1*, Matthew Bailey, Aidan A. Talorico, James Krehling, Kenneth Macklin, Dianna Bourassa, Poultry Science, Auburn University, Auburn, Alabama, United States.

Dust in poultry houses can contain high concentrations of airborne microorganisms and has potential to include pathogens from litter. Therefore, assessment of litter as a potential source of airborne pathogens is crucial. This study aimed at evaluating the potential for transfer of Salmonella Typhimurium, Escherichia coli, total coliforms, and aerobic bacteria from poultry litter with constant moisture level to air or dust in the poultry house. To evaluate this transfer potential, an in vitro system was developed. In a series of 2 L flasks, 110 g batches of poultry litter were inoculated with four levels of S. Typhimurium ranging from $10^2$ to $10^9$ CFU/mL and acclimated at room temperature for 24 h before sampling. After 24 h, 20 g of inoculated litter were removed to evaluate moisture and microbial count. Dust was then created by blowing air (143-155 m/s) in a direct stream onto the remaining 90 g of inoculated litter while simultaneously collecting dust by impingement in 10 mL buffered peptone water (0.8 L/min for 5 min). After sample collection, microbial analyses for Salmonella, E. coli, total coliforms, and aerobic bacteria were done for both litter and dust samples. Variability of litter moisture percentages were analyzed by one-way ANOVA and relationships between Salmonella litter levels and dust prevalence were analyzed by logistic regression. Inoculated litter moisture was consistent and ranged from 24.6% to 25.7% ($P=0.9603$). Following acclimation, Salmonella levels in litter decreased from initial inoculation levels by $1.51 \log_{10} \text{CFU/g}$ or to below the level of detection ($1.65 \log_{10} \text{CFU/g}$). Therefore, when dust samples were collected, litter Salmonella levels ranged from below the detectable limit (though positive with enrichment) to $6.16 \log_{10} \text{CFU/g}$ litter. Observed counts of E. coli, total coliforms, and aerobic bacteria in litter at sampling were $3.34, 4.29, \text{and } 10.57 \log_{10} \text{CFU/g}$, respectively. Salmonella and E. coli were below the level of detection ($1.1 \log_{10} \text{CFU/L of air}$) in all dust samples when directly plated. However, following enrichment, Salmonella positive dust samples were detected from litter containing $3.24, 5.17, \text{and } 6.16 \log_{10} \text{CFU/g}$, but not $2.18, 4.46$, or when levels were below $1.65 \log_{10} \text{CFU/g}$. Increasing Salmonella levels found in litter tended towards a Salmonella positive result in subsequent dust samples ($P=0.0714$). Coliforms were only detected in dust when the initial levels in the litter were at the highest level of $6.52 \log_{10} \text{CFU/g}$. All collected dust samples contained aerobic bacteria ($4.79 \log_{10} \text{CFU/L air}$). These results indicate potential for dust to be a source for Salmonella transmission.

Key Words: Salmonella Typhimurium, air, poultry litter, coliforms, dust

121 Evaluation of pulsed application of a water-administered Bacillus sp. fermentation product (Gamaxine) with and without antibiotic administration on broiler production parameters. Maria K. Dashek*, Jeffrey W. Hall1, Sherry L. Layton1*.1BV Science, Lake St Louis, Missouri, United States, 2Vetanco USA, Lenexa, Kansas, United States.

With the rise of antibiotic resistance, decreasing the use of antibiotics in animal agriculture has become important. Poultry producers are searching for innovative natural ways to improve animal production while humanely decreasing the reliance on antibiotics. The use of Bacillus subtilis and yeast as poultry probiotics is well-established due to the beneficial effects these microbes provide to intestinal health. It has been recently found that similar beneficial effects are appreciated by administering inactivated microbes and their fermentation products. Gamaxine is a combination of inactivated Bacillus sp., its fermentation products and yeast cell wall. In this study we evaluate the efficacy of Gamaxine to improve broiler production parameters in comparison and in conjunction with feed-administered antibiotics (FAA). This study was a 2 X 2 factorial completely randomized block design. Chicks were floor-raised with 30 broilers/pen, and fed diets with or without bacitracin methylene disalicylate at 50 g/ton to day 27 and Virginiamycin 20 g/ton on days 28-36 (FAA group), and were either administered water with or without 0.2 ml/bird of Gamaxine® via 1:128 water medicator on days 2 and 16 (n=6 pens). Chicks were challenged by top dressing the litter with recycled litter on day 4. Data collected included daily mortality pen weights and feed conversion ratio (FCR) on days 14, 28 and 36; data was analyzed using a two-way ANOVA. At the end of the trial, negative control birds (not administered Gamaxine or FAA) had an average weight of 1.977kg. Birds in the positive control (FAA with no Gamaxine administration) had an average weight of 2.156 kg. The difference between the negative and positive control illustrated that FAA had a beneficial effect on body weight without Gamaxine use. There was no significant interaction observed in end body weight, mortality or FCR. An overall 6% mortality rate was observed. FAA and Gamaxine had a significant main effect on body weight and Gamaxine had a significant effect on mortality adjusted FCR. FAA improved end body weight by 183 grams compared to birds fed the control diet ($p<0.05$). Birds not administered Gamaxine weighed 2.066kg, and the average body weight of birds administered Gamaxine was 2.155kg on day 36 ($p<0.0001$). Birds not administered Gamaxine in the water had a FCR of 1.537, the FCR was significantly improved with Gamaxine administration to 1.517 ($p<0.01$). In conclusion, both Gamaxine and FAA improved the average 36-day body weights. Gamaxine improved FCR, whereas FAA did not. In this trial Gamaxine improved broiler production parameters in the face of a recycled litter challenge similar to what is found in U.S. broiler barns.

Key Words: Bacillus subtilis, Broiler, Antibiotics, Yeast, Biopromoter
Improving broilers’ feed efficiency by dietary supplementation of a characterized natural citrus extract. Sekhou Cisse1*,2; Assia Boumezrag3, Roxanne Cornet4, Hoa Bui1,2, Mohamed el amine Benarbia1,2, David Guilet4, R&D, Nor-Feed SAS, Angers, Maine et Loire, France, 2R&D, Joint Lab ANR FeedInTech, Beaucouzé, Maine et Loire, France, 3R&D, University of Tiaret, Tiaret, Algeria.

Introduction: Plant extracts are more and more used in animal feed. Some of them such as citrus extract had already shown beneficial effect on animals’ performances and health. However, citrus extracts can vary in terms of concentration of active compounds. The lack of data concerning the composition make the results interpretation difficult. This trial was carried out in order to evaluate the effect of a Characterised Natural Citrus Extract (CNCE) in terms of composition and effect on performances.

Experimental design: Characterization of the CNCE (Nor-Spice® AB, Nor-Feed SAS, France) had been performed in triplicate. Three HPLC analysis methods were performed using HPLC-UV-DAD-DEDL (Shimadzu) and (HPLC UV-chromatographic systems. The main components of CNCE were identified using dereplication with Data Analysis software. In parallel, a trial was performed in an experimental farm in Algeria, using the same CNCE which have been characterized before. 360 one day old Arbor Acres as hatched were divided into 2 groups. Each group contained 6 replicates of 30 birds: Group 1: CTL group: Standard diet without supplementation; Group 2: CNCE group: Standard diet supplemented with 250 ppm of CNCE.

Litter was composed of a mixture of wood shaving and straw. Birds were reared until day 46 and were put under thermic stress at 30°C for 4 h per day, from day 26 to day 30. Zootechnical performances were weekly recorded.

Statement of statistical analysis: Statistical analysis were performed using R Studio software. Statistical significance was considered at p <0.05. Results: Spectral data from HPLC analysis allowed to identify approximately 30 secondary metabolites among carboxylic acids, phenolic acids, coumarin, flavones, flavanones and flavanols. Regarding zootechnical performances, no statistical difference was observed between the final weight of birds from CTL (2505 ± 41 g) and CNCE group (2517 ± 13 g).

Similarly, no difference was observed between feed intake, even if 150 g of difference was observed between the feed intake of CNCE group (5.46 kg/ broiler) and CTL group (5.61 kg/ broiler). No difference was also observed on water intake. However, the feed conversion ratio of broilers from CNCE group (2,198) was significantly lower (P = 0, 0395, T-test) compared to CTL group (2,285), translating a better feed efficiency from CNCE group. Conclusion: This study allowed to identify major molecules that enter the composition of CNCE. It also evidenced the positive effect of its dietary supplementation on broilers feed efficiency. However, more studies are necessary to clarify which compounds are responsible for the observed effects and their mechanisms of action.

Key Words: Citrus extract, Characterisation, Feed efficiency, Broiler chickens, Experimental trial

Allometric intestinal development of broilers from breeders of different ages in the pre-starter phase. Helder F. Oliveira1, Deibity A. Cordeiro1, Natiele F. Oliveira1, Carla Daniela S. Leite1, Imar C. Fernandes1, Jose H. Stringhini*1,2, Heloisa Helena d. Mello1, 2Zootecnia, Universidade Federal de Goiás, Goiania, Goias, Brazil, 1CNPq research fellow, Goiania, Goias, Brazil.

After hatch, it is common that neonate chicks have limited access to water and food for some hours or days. Because of this, large hatch windows should can result in dehydration in early hatched chicks which can hinder nutrient absorption, the poultry yolk sac retraction and consequently development of the digestive tract. An adequate intestinal development in hatching period will reflect in positive results in the subsequent phases of animal growth, therefore, it is important to highlight that the breeder age influences body development and performance. This experiment aimed to evaluate the effect of breeder age on intestinal development of chicks in pre-starter phase (1 to 7 days old). The experiment was carried out in University Federal of Goiás, Brazil. Broiler breeder eggs from different ages (39, 51 and 69 weeks) were incubated in a single stage incubator machine. The birds hatched were raised in a conventional shed with a rice straw litter in masonry boxes until 7 days of age, when these birds were euthanized and intestinal segments collected. An analysis of intestinal allometric development in relation to body weight and the total intestine length at 7 days was performed, using the following model: (p<0,05); in which: “Y” = organ variable (length of small intestine, duodenum, jejunum and ileum); “X” = live body weight, “α” e “β” = allometric coefficients. A natural logarithmic base was used to evaluate the relationships, and the classification of the “β” values for the different body components were: heterogenic (greater (hyper-allometric) or lower (hypoallometric) or isogonic (isometric) equal to 1 (one)), varying on a scale of, β<1, β>1 or β=1, having as final model, the following equation. In general, the results obtained showed that the intestinal development was not affected by breeder age, considering the body weight and the total small intestine length. But, chicks hatched from eggs of breeders with 69 weeks of age had for the total intestine length and ileum development observed a hypoallometric behavior (0.3419, confidence interval 0.0808 – 0.6029). In this case, it was observed a late development of the ileal segment in relation to the total intestine length basis. So, it is possible to conclude that the late intestinal development of the ileum in relation to the others parts of the intestine organs could bring a negative impact to the birds from older breeders.

Key Words: Allometric coefficient, breeder age, hatching eggs, intestinal development

Prevalence and risk factors associated with breast
white striping, woody breast and white striping) in broiler chickens from Ontario, Canada. Sunoh Che-GS*, Shai Barbut*, Chaoyue Wang*, Christian Fuchs1, Csaba Varga1, 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, 4Food processing plants for the presence of myopathies using a were evaluated from May 2019 to March 2020 at two random samples from each of 37 flocks (total 9,250 fillets) with these conditions in Canada. Two hundred and fifty causing economic losses. The aim of our study was to consumer acceptance and meat processability, ultimately determining the prevalence and assess risk factors associated with these conditions in Canada. Two hundred and fifty random samples from each of 37 flocks (total 9,250 fillets) were evaluated from May 2019 to March 2020 at two processing plants for the presence of myopathies using a multi-tier grading scheme for lesion severity. For each flock, we collected information regarding the environment (e.g., geographic location of growers, transport time), demographics (e.g., sex of broilers, average live weight of chickens), management (e.g., feed type, vaccination), and variables associated with plant operations (e.g., chilling method, truck hold time). Associations between these factors and the frequency of myopathies were tested using logistic and Poisson regression analyses. The prevalence of SM and severe WB was 35.9% (3,318/9,250, 95% CI: 34.9 to 36.9) and 11.8% (1,095/9,250, 95% CI: 11.2 to 12.5), respectively. Both SM and WB were present in approximately one-third of fillets. The prevalence of SM was significantly higher (P < 0.001) in a processing plant with water-chill (39.8%, 1,791/4,500, 95% CI: 38.4 to 41.3) instead of air-chill (32.1%, 1,525/4,750, 95% CI: 30.8 to 33.4). A higher (P < 0.001) prevalence of SM was observed in the summer [39.8% (1,890/4,750), 95% CI: 37.1 to 42.5] compared to the winter [18.9% (988/4,750), 95% CI: 16.6 to 21.4]), whereas a higher (P < 0.001) prevalence of severe WB was observed in the spring [26.6% (1,265/4,750), 95% CI: 24.3 to 29.2] compared to the fall [9.0% (429/4,750), 95% CI: 8.2 – 11.9]). Univariable logistic regression analyses showed that SM was positively associated with the average live weight of the chickens (OR = 2.01, P < 0.001, 95% CI: 1.44 to 2.81), and the average birds’ weight was 2358.81 ± 0.04 g. The prevalence of mild WS and moderate WS was 93.9% (8,684/9,250, 95% CI: 93.4 to 94.4) and 2.1% (191/9,250, 95% CI: 1.8 to 2.4), respectively, showing that almost all fillets exhibit some degree of white striping. In conclusion, these myopathies are a major concern for Ontario commercial broiler production, and some appear to be associated with seasons, meat chilling methods, and weight of the broilers.

Key Words: broiler myopathy, prevalence, spaghetti meat, white striping, woody breast

125 Intravenous T-61: an effective euthanasia method for poultry. Bethany Baker*, Antonietta L. Mortiz2, Danielle Zwueste*, Karen Schwean-Lardner1, Karen L. Machin1, Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 1Small Animal Clinical Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 2Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

T-61 is a non-barbiturate, non-narcotic euthanasia solution, consisting of embutramide (an anesthetic), mebenzonium iodide (a paralytic) and tetracaine hydrochloride (a potent local anesthetic). It is a potential method for euthanizing poultry when injected intravenously. However, at present there is no scientific literature evaluating the use and the appropriateness of T-61 for euthanizing birds. This study assessed the efficacy and efficiency of intravenous T-61 as a sole euthanasia agent for poultry, by euthanizing nine 5-week-old broilers via intravenous T-61 injection (dose = 0.03 mg/kg) and measuring the time to insensibility, brain death, cessation of audible heartbeat and abnormal electrocardiogram (ECG). Live focal sampling measured the latency to loss of nictitating membrane reflex (NIC) and palpebral blink reflex (PAL) to determine time to insensibility. Electroencephalograms were used to determine brain death, defined as the time to isoelectric activity. Auscultation determined cessation of audible heartbeat. ECG were also recorded and analyzed for each bird. When the ECG measured abnormal with a heart rate below 180, and the electroencephalogram was isoelectric, birds were considered dead (Coenen et al., 2000). Latency to NIC, PAL, cessation of heartbeat and isoelectric activity post-injection were compared via a paired T-test using PROC T-Test (SAS 9.4). Both NIC and PAL were lost 10.5 s post-injection, audible heartbeat disappeared after 24.5 s and isoelectric activity occurred 16.6 s after start of injection. Birds were all dead within 80 s of injection. Absence of NIC and PAL occurred before both the start of isoelectric activity onset (t0 = -6.00, P<0.01; t0 = -6.00, P<0.01, for NIC and PAL respectively) and cessation of audible heartbeat (t0 = -13.94, P<0.01; t0 = -13.94, P<0.01, for NIC and PAL respectively). There was a tendency for isoelectric activity to occur before audible heartbeat ceased (t0 = 8.70, P = 0.08). The rapid onset of insensibility means birds cannot experience pain or distress by 10.5 s from the start of the T-61 injection, and by 4 s from the end of the injection. As insensibility and irreversible brain death happened prior to cessation of heartbeat or abnormal ECG, birds were not conscious to cardiac and circulatory arrest. Furthermore, T-61 also irreversibly resulted in death. In conclusion, the intravenous injection of T-61 is an effective and efficient method of euthanasia for poultry.

Key Words: Brain Death, EEG, Euthanasia Solution, Insensibility, Avian
Reductions in dietary CP through the supplementation of feed-grade amino acids will allow for decreased dietary inclusion of soybean meal but previous experimentation has shown that the efficacy of reduced CP diets varies dependent upon diet composition. Therefore, two Experiments (Exp) were conducted to determine the efficacy of including wheat and corn at a 1:1 ratio in reduced CP diets. A High and Low diet was formulated to contain 23.5, 21.5, and 19.5% CP in the starter [2,940 kcal/kg; 1.28% digestible lysine (dLys)], grower (3,083 kcal/kg; 1.15% dLys), and finisher diets (3,130 kcal/kg; 1.02% dLys), respectively. The High and Low diets were then blended to produce a Medium diet containing 21.5, 19.5, and 17.5% CP in the starter, grower, and finisher diets. Starter, grower, and finisher diets were fed from 0 to 11, 12 to 27, and 28 to 41 d of age in Exp 1 (816 Cobb 500 broilers; 8 replicates; 34 birds/pen) and the grower diet was fed from d 8 to 28 in Exp 2 (108 Ross 308 broilers; 6 replicates; 6 birds/cage). Body weight gain, feed intake, and feed conversion ratio were determined for each experimental period in both Exp and diet AME, N retention, AMEn, and N digestibility were determined for an 8 to 28 d grower period in Exp 2. Data were subjected to a one-way ANOVA analysis and statistical significance was considered at \( P<0.05 \). No effects \( (P>0.05) \) of reduced CP were found on final BW, BW gain, or feed intake for any time point in Exp 1 but feed conversion was significantly increased \( (P<0.05) \) by approximately 3.5 and 4.4 points for broilers fed the Low diet compared to broilers fed the High and Medium diets for the 0 to 27 and 0 to 41 d periods, respectively. In E 2, broilers fed the Low diet displayed 0.166, 0.166, and 0.063 kg lower \( (P<0.05) \) final BW, BW gain, and feed intake, respectively, than those fed the High and Medium diets. Feed conversion increased \( (P<0.05) \) in a stepwise manner by 2.5 and 9.8 points as CP was reduced. Nutrient utilization assays found stepwise \( (P<0.05) \) increases in AMEn as dietary CP level was reduced and increased \( (P<0.05) \) N digestibility for broilers fed the High and Low diets compared to those fed the High. Live performance was sustained in the floor pen environment of Exp 1 with the exception of feed conversion. Nutrient utilization data from Exp 2 indicate that while gross nutrient utilization increases, these increases may be brought about by diet composition instead of as an internal regulation of metabolism to cope with reduced CP levels. These data indicate that diets using a 1:1 wheat and corn blend as the starch supplying grain allow for adequate performance in reduced CP diets when broilers are reared in floor pens.

**Key Words:** Reduced crude protein, Feed-grade amino acid, Maize, Broiler, Nitrogen digestibility

Reducing crude protein (CP) in broiler diets is a nutritional strategy which allows to partially replace dietary soybean meal (SBM) by cereals and which has proven benefits on litter quality, intestinal health and environmental impact. In addition, reduction of dietary SBM leads to a lower dietary electrolyte balance (DEB) due to the concomitant reduction of potassium (K) which can be detrimental to performance. The goals of this trial are therefore two-fold: to investigate 1) the effect of feeding broilers low CP diets and 2) the effect of DEB on animal performance, litter quality & foot pad dermatitis (FPD), blood parameters and carcass traits of broilers from 10 to 30 days of age. In total, 9,100 male Ross 308 chicks were randomly allotted to 35 pens of 260 broilers and housed in floor pens. They were fed a common diet from 0 to 10d and 5 dietary treatments from 10 to 20d and 20 to 30d. At 30d, 10 birds per treatment were slaughtered after a fasting of 12h. Experimental design consisted of 4 levels of dietary CP (control, -1%, -2% and -3%; T1-T4) with supplementation of K2CO3 to maintain a constant DEB level. An extra treatment (T5) was formulated at -3% CP without K2CO3 allowing DEB level to drop. All diets per feeding phase were iso-digestible Lys, iso-caloric and all indispensable AA levels were controlled above requirements. In diets with -3% CP (T4 & T5), SBM was fully replaced by wheat, potato protein and crystalline AA. All data was subjected to analysis of variance (ANOVA) using the SAS statistical package. Reducing dietary CP as low as 3% points and maintaining AA and DEB levels resulted in similar or better production performance from 10-30d for growth, feed intake and FCR (mean performance of 76g/d, 113g/d and 1.48, respectively). The reduction of dietary CP did not affect any carcass trait studied with mean breast yield of 31.4%. Daily water intake and water-to-feed ratio were decreased when lowering dietary CP \( (P<0.05) \). Triglycerides (\( P = 0.069 \)) and uric acid (\( P = 0.071 \)) content in serum tended to be higher and lower, respectively, in birds fed -3% CP. Excreta nitrogen, moisture and uric acid were significantly reduced in the -3% CP \( (P = 0.004, 0.013 \) and 0.002, respectively) while litter quality was improved.
(P = 0.002). When compared to T4, reducing DEB level at -3% dietary CP (T5) worsened FCR but further reduced water intake, water-to-feed ratio and FPD (P<0.001). Overall it can be concluded that supplementation of crystalline AA allows to totally replace SBM in broiler diets with -3% dietary CP with clear benefits on welfare and environmental impact. Although reducing DEB level in low CP diets worsened FCR, it also further improved FPD in this experiment.

Key Words: broiler, low CP, DEB, soybean meal, litter quality

128 Late vs. early planting/harvesting increased the energy and amino acid digestibility of faba bean cultivars fed to broiler chickens. Robin Ketelaar1, Miranda N. Smit1, Liangfei He1, Eduardo Beltranena1,2, 1Research and Extension Division, Alberta Agriculture and Forestry, Edmonton, Alberta, Canada, 2Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, Alberta, Canada, 3Wageningen University, Wageningen, Netherlands.

A concern of both pulse growers and poultry producers is how the timing of planting and harvesting can affect the feeding quality of faba bean. To investigate, two cultivars of zero-tannin (Snowbird, Snowdrop) and one cultivar of low vicine/convicine (Fabelle) faba bean sourced from certified seed growers were planted at a single site (53°38’52.2” N 113°21’09.2” W) EARLY or LATE May and desiccated mid-September (EARLY) or early October (LATE), respectively, to purposely increase the proportion of damaged beans at harvest. EARLY and LATE harvested beans of each cultivar and a N-free diet were then fed to 756 broiler chickens (Ross 708) housed in 56 floor level pens in a 2 x 3 factorial design (7 pens/diet of 13-14 birds) plus N-free diet (14 pens of 13-14 birds). For d 0–14, broilers had access to commercial starter diets, after which 42 pens were switched to 1 of 6 test diets (95% faba bean inclusion). Ileal digesta was collected after euthanizing individual birds the last week of the experiment (53). For d 15–21, all birds were switched to the experimental diets. Birds consumed high-lysine (1.42% total lysine) or low-lysine (0.85% total lysine) diet while the control group consumed 1.14% total lysine diet. These concentrations of lysine represent 125% (high lysine), 75% (low lysine) and 100% (control) respectively, of National Research Council (NRC) recommendation for broiler chickens. Tissues were collected at 8 weeks of age after birds were fasted for 12 hours. After comparing the low and high lysine groups, 210 differentially expressed genes (DEGs) were identified (fold change >1 and false discovery rate (FDR) < 0.05). There were 67 up-regulated genes and 143 down-regulated genes among these DEGs. Analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analysis show that cellular growth, lipid metabolism, lysine metabolism pathways were among the significantly enriched pathways. Highly DEGs in these pathways include Aminoadipate-Semialdehyde Dehydrogenase-Phosphopantetheinyl Transferase (AASDHPP), Aminoadipate-Semialdehyde Synthase (AASS), Solute Carrier Family 26 Member 6 (SLC26A6), Fibroblast Growth Factor 1 (FGF1) and (Solute Carrier Family 7 Member 1 SLC7A1), among others. This study provides a better understanding of the pathways that may be...
associated with lysine uptake and metabolism in broiler chickens. Some of the DEGs obtained in this study may also be utilized as lead in the search for candidate genes that may be associated with lysine uptake and metabolism in broiler chickens.

**Key Words:** Lysine, Poultry, transcriptome analysis, amino acid transporters

130 **Suppressive effects of glutamate and glutamine co-supplementation on muscle proteolysis in heat-stressed broiler chickens.** Kyohei Furukawa1, 2, Motoi Kikusato1, Wu Guoyao1, Masaaki Toyomizu1, 1Graduate School of Agricultural Science, Tohoku University, Sendai, Japan, 2The University of Tokyo, Bunkyo-ku, Tokyo, Japan, 1Department of Animal Science, Texas A&M University, College Station, Texas, United States.

Heat stress (HS) induces proteolysis in the skeletal muscle of chickens. Our previous study indicated that HS increased the mRNA level of atrogin-1, which is muscle-specific ubiquitin ligase, at the early stage. During the same period, we observed an increment in the production of mitochondrial reactive oxygen species (ROS) and the downregulation of insulin signaling pathway in the muscle. We hypothesized that these metabolic defects are the targets to control HS-induced muscle proteolysis. Because glutamate (Glu) and glutamine (Gln) play important roles in oxidative stress and insulin signaling, we investigated the suppressive effects of Glu/Gln co-supplementation on muscle proteolysis in HS-treated chickens. Forty-one 22-days-old male broilers (ROSS 308) were exposed to thermoneutral (TN; 24 °C) or HS (34 °C) conditions; the birds were fed a diet supplemented with either 1.83 % alanine (iso-nitrogenous control) or 1% Glu plus 1% Gln. After 0.5 d of HS exposure, the *pectoralis major* muscles and plasma samples were excised (TN, 7 birds; HS and HS + Glu/Gln, 8 birds/ group), and plasma samples were collected after 3 d of the exposure (TN and HS, 5 birds; HS + Glu/Gln group, 8 birds/ group). Chickens were not fasted prior to the sample collection. The plasma concentrations of insulin and 3-methylhistidine (the indicator of muscle proteolysis) were quantified by the ELISA kit and HPLC. Atrogin-1 mRNA levels and the phosphorylation ratio of Akt and FoxO1 were determined by RT-PCR and western blotting. MDA contents were determined with a colorimetric method to evaluate intramuscular oxidative stress. Data were analyzed by the Tukey-Kramer multiple comparison test and values < 0.05 were taken to indicate statistical significance. Under HS conditions, atrogin-1 mRNA levels in the muscle and 3-methylhistidine concentrations in the plasma were increased (P < 0.05) at 0.5 and 3 d, respectively, when compared with the TN conditions. These increments were reduced by the Glu/Gln co-supplementation (atrogin-1, P = 0.07; 3-methylhistidine, P < 0.05). MDA contents in the muscle were increased after 0.5 d of HS (P < 0.05), but it was restored by the Glu/Gln co-supplementation (P < 0.05). HS decreased the phosphorylation levels of Akt (P < 0.05) and FoxO1 (P = 0.06) in the muscle, but those were partially ameliorated by the Glu/Gln co-supplementation (P > 0.10 vs TN) exhibiting intermediate levels between TN and HS groups. In contrast, HS lowered insulin concentration in the plasma, that was kept in the Glu/Gln group (P < 0.05 vs TN). This study suggests the suppressive effects of Glu/Gln co-supplementation on HS-induced muscle proteolysis may be mediated via mitigating oxidative stress and Akt-FoxO1 phosphorylation.

**Key Words:** Heat stress, Skeletal muscle, Atrogin-1, Oxidative stress, Insulin signaling pathway

131 **Composition of different feather tracts of broilers.** Bruno Balbino Leme1, 2, Carolina Cardoso Nagib Nascente1, Matheus D. Reis1, Larissa Vargas1, Rony Riveros Lizana1, Robert Mervyn Gous3, Nilva Sakomura4, 1Animal Science, UNESP, Jaboticabal, São Paulo, Brazil, 4University of KwaZulu-Natal, KwaZulu- Natal, South Africa.

The aim of this study was to evaluate the water and protein in broiler’s feather during growth and estimate the modifications on these variables in seven distinct feather regions. Two-hundred 1d old Cobb 500 MX broilers chick were allocated in 10 pens of 20 birds. From 14 to 112d, every two weeks, 10 birds were randomly selected and euthanized for feathers sampling, which was performed delimiting the feather cover in seven tracts (dorsal-pelvic (Dpe), interscapular (Int), capital-cervical (Ccer), pectoral (Pec), femoral (Fem), humeral-alar (Hal) and dorso-caudal (Dca)) and saving the feathers separately by tract, for further analysis. After sampling, feathers were grounded using a multipurpose mill and analyzed for dry matter and nitrogen content, according to AOAC methods. Water content (g/kg) was calculated subtracting the weight of feather by the dry matter content and crude protein (g/kg) was obtained multiplying nitrogen content by 6.25. Simple linear regression analyses were conducted to determine the change in protein and water contents over time for the feathers from each of the seven tracts. The constant terms and slopes from each tract were compared using simple linear regression with groups in Genstat 18th edition (VSN International, 2016) using the Dpe as the reference tract. For all tracts, the water content decreased with time (P < 0.05), however, the rate of decline was the same (-3.444, P>0.05) for the Dpe, Int, Dca, and Fem tracts. The rate of decline for water content estimated for Pec, Ccer, and Hal tracts was estimated as -2.878, -2.696, and -2.459, respectively. The initial amount of water was the same (605 g/kg, P>0.05) only between Dpe and Fem tract. The values estimated for Int, Dca, Pec, Ccer, and Hal tracts was estimated as 638.9, 649.4, 583, 551.9, and 539.7 g/kg, respectively. The protein content of feathers increased significantly with time, especially for Dpe (0.78) and Int tracts, with no difference between them (P>0.05). The estimated values for Dca, Fem, Pec, Ccer, and Hal was -3.988, -3.738, -3.844, -3.881, and -3.883, respectively. The initial protein content was estimated as 900, 614.6, 629.5, 632.4, 638.3, and 649.4, 583, 551.9, and 539.7 g/kg, respectively.
647.9 g/kg, respectively for Dpe, Int, Dca, Fem, Pec, Ccer, and Hal tracts. The results provide evidence that the growth of feathers is not uniform between the tracts of broilers. Thus, since the amino acid requirements for feather growth have always been considering feather as a whole keratinized tissue, the amino acid requirement for feather development may have been underestimated and further studies might be able to better estimate amino acid requirements for improved development of broiler feathers.

**Key Words:** Protein, Water

132 Wnt/β-catenin signaling pathway mediated methionine promotes feather follicle development in chick embryos. Meng-jie Chen*, Xiuli Wang, Hui-chao Yan, Chun-qi Gao, College of Animal Science, South China Agricultural University/Guangdong Provincial Key Laboratory of Animal Nutrition Control, Guangzhou, Guangdong, China.

Feathers play a critical role in conserving heat, which directly affects productivity. Whole-body feathers also affect commodity value based on the appearance of live poultry and the uniformity of a carcass. Previous studies have shown that dietary supplementation with methionine (Met) promotes the growth of feathers. However, the regulatory role and the molecular mechanism of Met in feather follicle development and feather growth of chicks as well as the difference in effect between L- and DL-Met are still unclear. This study used chick embryo (Qingyuan partridge chick) as a model to explore the regulatory role of Met in feather follicle and feather development. A total of 280 fertile eggs (40 eggs/group) were respectively injected with 0, 5, 10, and 20 mg L-Met or DL-Met/egg on embryonic day 9 (E9), samples were collected on the E15 and day of hatching (DOH). The whole-body feather masses were determined to describe the feather growth, and skin samples were detected by H&E staining to evaluate feather follicle development. The total protein in the skin was extracted to detect β-catenin, c-Myc, TCF4, and Cyclin D1 (Cell Signaling Technology Beverly, MA, USA) levels by Western blotting. Data were subjected to one way ANOVA (Cell Signaling Technology Beverly, MA, USA) levels by Western blotting. Data were subjected to one way ANOVA (SAS 9.2). Results showed that the in ovo injection of L- or DL-Met did not affect embryo weight (P > 0.05). The injection of 5 and 10 mg/egg L-Met or DL-Met significantly increased the feather weight at E15, and injection of 10 mg L-Met, or 5 and 10 mg/egg DL-Met significantly increased the feather weight on the DOH (P < 0.05). Meanwhile, the main effect analysis suggested that changes in the weight of embryos and feathers were related to the injection of Met levels (P < 0.05) but not to the Met source (P > 0.05). The injection levels of L- and DL-Met were quadratically correlated with the feather weight of chicks (P < 0.05). Correspondingly, all injection of L- or DL-Met doses significantly increased the diameter and density of feather follicle on the DOH, as well as the protein expression level of β-catenin, TCF4, Cyclin D1, and c-Myc on E15 and the DOH, especially the 10 mg/egg dose (P < 0.05). In conclusion, the injection of either L- or DL-Met, especially the 10 mg/egg dose, can improve feather follicle development by activating Wnt/β-catenin signaling, thereby promoting feather growth. There is no difference between L- and DL-Met treatments on feather development. Funding for this project was provided by the National Natural Science Foundation of China (31972585), and the National Science Foundation of Guangdong Province, China (2018B030315001).

**Key Words:** feather development, feather follicle, methionine, Wnt/β-catenin signaling

133 Influence of diet formulation technique on requirements and ideal profile of digestible Lys, Met+Cys and Thr for grower of Japanese quail. Tamires M. Silva Felix*, Aliton N. Silva, Thalis Oliveira, José H. Vilar Da Silva, Animal Science, Universidade Federal da Paraíba, Solânea, Paraíba, Brazil.

Three separate experiments were conducted with Japanese quail of 10 to 35 days of age to determine the influence of graded supplementation method and dilution technique on estimates of requirements and ideal profile of Met+Cys and Thr relative to Lys for the maximum grower. All the birds were weighed individually at 10 days of age to calculate the average weight the lot of 672 in trial 1 (33.7 ± 0.42 g LW), 588 in trial 2 (34.02 ± 1 g LW) and 588 in trial 3 (33.05 ± 1 g LW), and after, were completely randomized allotted in a factorial scheme 2x7 (two formulation techniques, seven levels of each amino acids) and 14 dietary treatments per trial and 6 repetitions of 8 birds (trial 1) and 7 birds (trial 2 and trial 3). The levels of DLys were 0.70; 0.80; 0.90; 1.00; 1.10; 1.20 and 1.30%; DMet+Cys were 0.46, 0.58, 0.70, 0.82, 0.94, 1.06 and 1.18%; and DThr were 0.55, 0.67, 0.79, 0.91, 1.03, 1.15, 1.27%. For the graded supplementation method, three basal diets first-limiting only DLys (trial 1), DMet+Cys (trial 2) and DThr (trial 3) were supplemented, respectively, with L-Lys•HCl (78.4%), DL-Met (99%) and L-Thr (99%) to replace the cornstarch, obtaining 7 isonenergetic and isonitrogenous diets. For dilution technique, diets were formulated for each trial, by diluting a concentrated (summit) mix with dilution mixture. In all experiments the dilution mixture was protein-free. The summit diet in each trial was designed to provide lysine at between 1.3, Met+Cys 1.53, and Thr 1.48 times, and the other amino acids at not less than 1.70 to 1.86 times (Trial 1), 2.56 to 2.89 times (trial 2) ad 2.30 to 2.44 times (trial 3) the requirements of the Japanese quail of 10 to 35 days of age. Experimental diets by dilution technique were made by blending summits mix and dilution mix em different proportions to obtain similar levels of amino acid tests content of graded supplementation diets. The data were submitted to the Tukey test and quadratic polynomial function (P <0.05), using the SAS software (Version 9.1). The requirement of DLys were 1.05% or 262 mg and 1.14% or 285 mg, Met + Cys 0.82% or 205 mg and 0.85% or 250 mg and Thr 0.82% or 205 mg and 0.96% or 240 mg/day,
respectively, for graded supplementation method and dilution technique. The ideal profiles were 100% Lys, 78% Met + Cys and 78% Thr by graded supplementation method and 100% Lys, 74% Met + Cys, 84% Thr by dilution technique. The tendency to overestimate the requirements of amino acids and unbalance ideal profile Met+Cys and Thr relative Lys are majors disadvantages of the dilution technique compared the graded supplementation method, namely that of the crude protein-energy balance changing systematically in successive dietary treatments.

Key Words: Amino acids, Diet formulation techniques, Ideal protein

134  Determination of the 4th limiting amino acid for Ross 308 male broilers from 15 to 35 day in low crude protein wheat-based diets. Craig W. Maynard1,2,3, Michael T. Kidd1, Peter V. Chrest2, Peter H. Selle2, Sonia Liu2, Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, 2Poultry Research Foundation, The University of Sydney, Camden, New South Wales, Australia, 3Baiada Poultry Pty Limited, Pendle Hill, New South Wales, Australia.

As more feed-grade amino acids are brought to the market at economically viable prices, they are implemented into broiler diet formulation, reducing dietary CP in broiler feeds. Formulation modeling has been implemented to test which amino acids will become next limiting and will need supplementation in feed-grade form, but these hypothesized amino acids need to be validated experimentally. Therefore, an experiment was run to determine the 4th limiting amino acid in wheat-based broiler diets. An industry grower diet (IC; 21.0% CP; 12.9 MJ/kg) was formulated to be representative to Australian grower diets. Dietary CP was subsequently reduced by 3.0 percentage points and energy content reduced by 0.5 MJ/kg, creating the negative control (NC). The NC diet was then supplemented with feed-grade Val, Ile, Leu, Trp, Arg, His, Phe+Tyr, Gly+Ser, and Pro to match the amino acid profile of the IC, soybean oil was also added to supply 0.5MJ/kg to resemble the IC, resulting in the positive control (PC). Supplemented amino acids and energy were then individually serially deleted forming 10 experimental diets identified by their deleted amino acid or energy, resulting in 13 total experimental treatments. All experimental diets contained 1.15% digestible Lys, 0.87% digestible TSAA, and 0.77% digestible Thr and all diets were isonitrogenous. The 13 experimental diets were fed to six replicate pens of 12 birds (78 pens; 936 broilers). Body weight gain, feed intake, and feed conversion were determined for a 15 to 35 d grower period and on d 35, five broilers per pen were deboned to determine total breast, right thigh, and fat yield. Data were subjected to ANOVA analysis and statistical significance was considered at P≤0.05. Broilers fed the IC and PC performed and yielded similarly (P=0.05) for all recorded measurements, while removal of all feed-grade amino acids and energy resulted in reduced (P<0.05) performance and breast yield for broilers fed NC diets. Of the amino acid deletion diets, the removal of Val resulted in the lowest (P<0.05) BW gain, the removal of Val and Ile resulted in the highest (P<0.05) feed conversion, and the removal of Val, Ile, and Leu resulting in the lowest (P<0.05) total breast yield. Interestingly, the removal of energy from the diet resulted in BW gain superior (P<0.05) to the IC diet with similar (P>0.05) feed conversion and total breast yield. The 4th limiting amino acid in wheat-based diets appears to be Val, although all three of the branched-chain amino acids influenced key parameters for poultry profitability. Reducing dietary energy may result in improvements in poultry performance without impacting total breast yield.

Key Words: limiting amino acid, broiler, performance, carcass traits

135  Determination of standardized digestible amino acid requirements for broiler breeders. Jordan Weil1,2, Antonio Betitia1, Nawin Suesuttajit1, Katie Hilton1, Pramir Maharjan1, Diego Martinez2, Justina V. Caldas3, Shivi Rao1, Craig N.Coon1,1Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, 2DuPont, Wilmington, Delaware, United States, 3Cobb-Vantress Inc., Siloam Springs, Arkansas, United States.

Evaluating amino acid (AA) requirements is essential in order to ensure optimal egg mass, fertility, and performance. Not only can AA vary across environments, breeders, genders, ages, and body composition, but the method in which such requirements are estimated can influence estimations. To avoid erroneous estimates, standardized ileal digestibility (SID) techniques can be implemented to minimize error. This is satisfied by accounting for endogenous losses or the bioavailability of nutrients in provided feedstuffs. In this experiment, SID methods were used to determine the digestible AA requirements in Cobb 700 FF broiler breeders. A total of 648 21 wk-old Cobb 700 FF broiler breeders were placed into 648 cages, with each cage containing one breeder. A total of eight test AA were studied in this experiment (10 reps/diet). Test AA included the total sulfur amino acids (Met + Cys), lysine (Lys), arginine (Arg), branched chain AA (Val, Ile), threonine (Thr), tryptophan (Trp), and glutamic acid (Glu). The concentration of AA that were fed to all treatments except for the test AA was equivalent to 120% Primary Breeder recommended AA intake (Cobb-Vantress, 2012) for peak production. Birds were fed for a period of 7 weeks (2 wks adaptation; 5 wks testing) in two phases. Phase I (P1) lasted from wks 30 through 37, while Phase 2 (P2) occurred from wks 44 through 51. Results were analyzed using quadratic, broken line, and quadratic broken line models (JMP® Pro 15). Quadratic AA requirements for egg mass were as follows: Met+Cys, Lys, Arg, Ile, Val, Thr, and Trp are 880, 900, 900, 787, 767, 672, and 256 mg/day, respectively, for P1, and 803, 1110, 933, 811, 811, 681, and 235 mg/d for P2. Digestible AA requirements as determined through the broken line model were 608, 833, 562, 533, 650, 451, and 214 mg/d for Met+Cys, Lys, Arg, Ile, Val, Thr, and Trp,
across, during P1 and 556, 1095, 638, 559, 723, 645, and 162, respectively, during P2. Digestible requirements as determined through the quadratic broken line model were as follows for P1 Met+Cys, Lys, Arg, Ile, Val, Thr, and Trp: 729, 885, 628, 779, 732, 542, and 241 mg/d, and for P2, 658, 1105, 662, 708, 776, 670, and 187 mg/d, respectively. Each statistical method is useful on its own right as producers choose to increase or decrease egg weights contributing to overall egg mass. The digestible protein inclusion level was determined to be 21 and 19 (P1 and P2) g/d by feeding a series of glutamic acid levels plus digestible essential amino acids needed for optimum egg mass production. Knowledge gained from this study will provide the necessary information to determine the production requirements of Cobb 700 broiler breeders for more accurate diet formulations.

Key Words: broiler breeders, amino acids, requirements, standardized ileal digestibility

136 Determining the lysine requirement in broiler breeders using the indicator amino acid oxidation and nitrogen balance methods. Jordan Weil1, Antonio Beitia1, Nawin Suesutaitij1, Katie Hilton1, Pramir Maharanj1, Diego Martinez1, Justina V. Caldas2, Shivi Rao2, Craig N. Coon3, ‘Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, 1Cobb-Vantress Inc., Siloam Springs, Arkansas, United States, 2DuPont, Wilmington, Delaware, United States.

Over time, the need update amino acid requirements for broiler breeders is increasingly important in order to ensure optimal egg production, hatchability, and progeny performance. Amino acid requirements can be determined through the nitrogen balance method, standardized ileal digestibility (SID), or indicator amino acid oxidation (IAAO). The latter involves placing birds into metabolic chambers for measurement of amino acid oxidation. Each method has advantages and disadvantages. For example, the IAAO method is minimally invasive, but is costly due to the involvement of respiratory chambers and an indicator amino acid which is typically in the form of a stable isotope. The trade-off of expenses for the rapid determination of amino acid requirements is useful for young chicks, diseased animals, and animals in production. In this experiment, a total of 24 birds 55 wk-old Cobb 700 FF broiler breeders were placed into 12 metabolic chambers, with each chamber containing one breeder. Lysine requirements were determined twice, requiring a total of 24 birds (12 birds/round). Six diets with varying levels of Lys were utilized for this experiment. All amino acids excluding Lys were included at 20% above the primary breeder requirement. The control diet was fed for two days, followed by a day in which the test diet was fed, a tracer amino acid was supplied, and breath samples were collected. On test day, a priming dose of L-[1-13C]phenylalanine (Cambridge Isotope Laboratories, Inc.) based on the subject’s body weight was first supplied, followed by [1-13C]Phe doses every thirty minutes, spanning a four hour period. Respiration samples were taken every thirty minutes. Respiration samples were taken every thirty minutes. Respiration samples were taken every thirty minutes. Respiration samples were taken every thirty minutes. Results for IRMS were converted to atom percent excess (APE) and analyzed using a piecewise model of best fit (JMP® Pro 15). Through the piecewise regression, the digestible Lys requirement for broiler breeders was determined to be 908 mg/day. In comparison with a digestibility trial in which 70 Cobb 700 hens were fed diets containing varying levels of Lys over a seven week period (2 wks adaptation; 5 wks testing), the IAAO estimation was slightly higher, but comparable, to the estimated broken line requirements for standardized digestibility. The digestible requirement for egg mass was estimated at 833 and 900 mg/day for a broken line and quadratic estimations, respectively. Knowledge gained from this study is useful as the poultry industry strives to quickly determine amino acid requirements in a safe manner.

Key Words: amino acid requirement, stable isotopes, indicator amino acid oxidation, standardized ileal digestibility, broiler breeder

137 Response of laying hens to the intake of digestible leucine. Mirella Melaire1, Matheus D. Reis, Felipe Fabbri, Guilherme Teofiló, Nilva Sakomura, UNESP, Jaboticabal, Sao Paulo, Brazil.

The objective of this study was evaluate the response of laying hens consuming diets with levels of digestible leucine (dLeu) and estimate the level based on maximum response. A total of eight treatments were randomly distributed in 120 experimental units of one bird each (Lohmann - LSL Lite of 63 weeks-old), performing fifteen replication per treatment. A summit diet was formulated to contain an excess of all essential amino acids, but dLeu was kept in relative deficiency (30%) to the recommended ideal ratio with digestible lysine (Brazilian Tables for Poultry and Swine, 2017). The summit diet was blended with a nitrogen-free diet to produce seven dietary treatments with crescent levels of dLeu (6.0; 7.0; 8.0; 9.0; 10.0; 11.0; 12.0 g of dLeu /kg of feed). The diet containing the lower level of dLeu was used to produce the counter proof, adding L-Leucine to free diet to produce seven dietary treatments with crescent levels of dLeu (6.0; 7.0; 8.0; 9.0; 10.0; 11.0; 12.0 g of dLeu /kg of feed). The diet containing the lower level of dLeu was used to produce the counter proof, adding L-Leucine to reach the level of 7.0 g of dLeu / kg of feed. The experimental diets were offered to the laying hens during ten weeks, but only the last four weeks were used for statistical analysis. Feed intake (FI, g/bird/day), egg production (EP, %), and egg weight (EW, g) were weekly measured. dLeu intake (FI x dLeu, mg/bird/day) and egg mass (EP x EW, g) were calculated. The response variables were submitted to ANOVA, considering 5% of probability. A Dunnet test was used to verify if the counter proof was statistically different from treatment one. To estimate the responses of laying hens, dLeu intake was used as independent variable to predict EP, EW, and EM according to three models: linear quadratic (QUAD), linear broken-line (LBL), and quadratic broken-line (QBL), according to Robbins et al. (2006). The Akaike information criterion
(Akaike, 1974) was used to evaluate the goodness of fit between models. The results demonstrate that dLeu was the first limiting nutrient in the feeds, since the counter proof group demonstrates an improvement on EP, EW, and EM compared to lowest level (P < 0.05). There was statistical difference between laying hens consuming crescent levels of dLeu for all response variables evaluated (P < 0.05). The lower value of AIC estimated was obtained when QUAD model was used to predict the response variables EP (EP = 11.15+0.14*dLeu-0.00006*dLeu²) and EM (EM = -5.55+0.18*dLeu-0.00005*dLeu²), whereas for EW the LBL demonstrates the lower AIC value (EW = 65.19-0.015*(937.86-dLeu)). dLeu level to maximize EP, EW, and EM was estimated to be 11.56, 9.38, and 12.06 g of dLeu/kg of feed, respectively.

**Key Words:** amino acid, egg mass, egg production

**138 Meta-analysis of broiler response to dietary valine supply.** Maroua Zouaoui*, William Lambert¹, Marie-Pierre Létourneau-Montminy², ¹Sciences Animales, Université LAVAL, Quebec, Quebec, Canada, ²Ajinomoto Animal Nutrition Europe, Paris, France.

Low crude protein (LCP) diets is an efficient strategy to decrease environmental footprint, but a precise knowledge of the requirement of indispensable amino acids (AA) is needed. Valine (Val) has been identified as the fourth limiting AA in corn soybean meal broiler diets. Many dose response studies of Val have been performed to estimate standardized ileal digestible (SID) Val (ValSID) requirement. Requirement vary due to the use of different basal diet nutritional composition, genetic, ages and statistical models. Meta-analysis appeared suitable for a more precise estimate of Val requirement. The purpose of this study was to estimate the Val requirement of broilers through a meta-analysis approach performed based on the significant response of broilers to Val supply. The database is made up of 23 publications published between 1999 and 2018, reporting 44 trials for a total of 229 feeding treatments of male chickens with at least 4 levels of Val. The ValSID were recalculated from INRA Tables (2018). The X axis (requirements) for Val are expressed relative to that of Lysine (Lys) on a SID basis (SID Val:Lys). Growth performance criteria were Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed Conversion Ratio (FCR) expressed in percentage relative to the highest value (100%) to make the values comparable between age and genetic line. In a specific trial, when ADG or FCR responded significantly to dietary Val with a linear-plateau or a curvilinear-plateau model, the trial was positively selected, as previously described by Van Milgen et al. 2012. This results in the selection of 26 Val dose-response studies among the 44, whose genetic lines are Ross (70%) or Cobb (30%) with two thirds being growing animals and the rest in finishing. A linear-plateau (LP) and a curvilinear-plateau (CLP) models were fitted with PROC NLIN procedure of SAS 9.4 with the random effect of the experiment to describe the response of ADG, ADFI and FCR to increased SID Val:Lys. The estimated SID Val:Lys requirements maximizing ADFI, ADG, and FCR were respectively, 71, 78, and 79% using a linear-plateau model (LP), and 77, 87, and 94% using a curvilinear-plateau model (CLP). The coefficient of determination (R²) ranged from 0.50 for ADG LP to 0.72 for FCR LP and CLP. This study showed that for growth performance criteria SID Val: Lys requirement estimates are found to be greater with a CLP than with a LP model, and for FCR. The results of this meta-analysis will facilitate the implementation of LCP strategy without affecting broiler performance.

**Key Words:** Broiler, ADG, ADFI, FCR, Valine
139  Effect of feeding inositol or high phytase on laying hen performance and amino acid digestibility. Eugenia Herwig*1, Carrie Walk2, Mike Bedford2, Henry Classen1, Karen Schween-Lardner1, ‘Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 2AB Vista, Marlborough, United Kingdom.

The products of the complete de-phosphorylation of phytate are phosphorus and inositol. High levels of phytase are reported to improve broiler performance and amino acid digestibility, especially with reduced protein level diets. Some of this improvement is attributed to the release of inositol, but little is known about its effects on laying hens. To assess the effect of high levels of phytase or the equivalent amount of inositol released by phytase action on laying hen performance and amino acid digestibility, five diets were formulated: high balanced protein (HBP- 840 mg of Dlys/h/d), HBP with inositol (HBP+I – 672 mg of Dlys/h/d), reduced balanced protein (RBP – 16% crude protein), RBP with phytase (RBP+P – 3000 FTU) and RBP with inositol (RBP+I – 0.16%). Each diet was fed, in a completely randomized design, to 72 Lohmann LSL -lite laying hens (6 hens/cage; 503 cm²/bird) and feed and water were provided ad libitum. Data were analyzed with a one way ANOVA and four orthogonal contrasts to assess the effect of protein (HBP+HBP+I vs. RBP+RBP+I), inositol (HBP+RBP vs. HBP+I + RBP+I), phytase (RBP vs. RBP+P), and phytase vs. inositol (RBP+P vs. RBP+I). Significance was set at 0.05 and interpretation was based on contrast results. Neither protein nor phytase affected laying hen performance. Inositol increased feed intake (117 vs. 118 g/day, P = 0.03), but diet did not affect egg production (average = 89.8%) or feed efficiency (average = 2.15 g/g). No differences were found for the percentage of unsalable eggs (average = 0.5%), but inositol increased the incidence of abnormally shaped eggs (0.05 vs. 0.21%, P = 0.01) and reduced eggshell quality (specific gravity 1.088 vs. 1.087, P < 0.01). Also, inositol tended to increase overall hen mortality (5.6 vs. 18.8%, P = 0.07). The amino acid digestibility of six indispensable (P < 0.05) and four dispensable amino acids (P < 0.02) decreased in RBP vs HBP diets, while phytase reduced methionine digestibility (0.91 vs. 0.88, P = 0.05). No effect of inositol on amino acid digestibility was found. In conclusion, unlike in broilers, inositol or phytase supplementation did not improve hen production performance or amino acid digestibility in this experiment. What is more, dietary inositol might have a negative effect on egg quality.

140 Phytase on leg health parameters and bone development under commercial conditions for Ross 708 broilers. Maria C. Alfaro-Wisaquillo*1,2, Andres Ortiz1,2, Yilmar Matta1,2, Sebastian Hoyos1,2, Ghery D. Buitrago1,2, Juan D. Martinez1,2, Jonathan Yanquen1,2, Anel Atencio1, Rocio Crespo1, Edgar O. Oviedo-Rondon1, ‘Prestige Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, United States, ‘College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, United States, ‘Perdue Foods, LLC, Salisbury, Maryland, United States.

Leg problems are still the leading cause of broiler culling and a significant animal welfare concern. During the last decades, the adequate use of phytase has played a key role in decreasing these problems by improving bone development and mineralization. Though, there are concerns that sporadic issues related to mixing and enzyme recovery post pelleting could negatively affect leg health. The objective of this study was to evaluate the effects of phytase supplementation on leg health parameters like angular deformities, tibial (TD) and femoral dyschondroplasia (FD), footpad dermatitis (FPD), hock burns (HB), leg bone relative asymmetry (RA), tibia weight and thickness, breaking strength, and ash content on chickens at 28d. Eight commercial farms were selected to randomly assign two treatments, phytase supplementation, 1,500 FTU/kg (3X), and diets with inorganic phosphate-no phytase, to obtain 4 farms/treatment with 4 houses each. The experimental unit was the house. The phytase replaced the dietary equivalent to 0.15% P and 0.17% Ca. Adequate phytase recovery was confirmed. All chickens were fed standard starter and grower diets that only varied on phytase content. At 28d, 100 chickens/farm, 25/house were selected for leg health assessment and 16 chickens/farm, 4/house for tibia sample collection. Likewise, 8 chickens/farm (4 valgus, 4 straight) were selected for FD evaluation. Femurs were collected, decalcified, fixed, and sectioned at 4 µm to be stained with HE. The length and area of femur growth plates were measured. Data were analyzed in a completely randomized design using ANOVA with houses nested within farm and farms nested within dietary treatments. Mean separation was conducted using a t-test. Correlation analysis was performed in TD and FD evaluation. The incidence of valgus angulations was higher than 30% at 28 d of age at all farms. However, no significant effects (P>0.05) due to treatments were observed in leg health parameters, RA, or thickness. Phytase supplementation improved (P<0.05) dry bone weight, tibia breaking strength, and ash content. Correlations (P<0.001)
were observed between chicken weight, length, and area of the tibia growth plates. The effects of farm were detected (P<0.05) in the incidence of angular deformities, FPD, tibia thickness and dry bone, indicating that house environmental conditions and/or quality of chicks were more important than diet in leg health and affected bone development. No significant effects (P>0.05) of dietary treatments or farm conditions were observed in HB, relative length RA or relative weight. In conclusion, phytase supplementation did not significantly affect leg problems and improved bone mineralization and strength.

**Key Words:** Phytase supplementation, Bone development, Tibial dyschondroplasia, Ash content, Leg health


An experiment was conducted to compare the efficacy of an intrinsically heat-stable phytase (OP; OptiPhos Plus, Huvepharma, Peachtree City, GA) with a commercially available coated phytase (OP; OptiPhos 6000PF, Huvepharma, Peachtree City, GA) in their ability to improve the growth performance and tibia characteristics of broilers fed a P and Ca deficient basal diet. Male by-product chicks from a Cobb 500 female line were reared in battery cages and provided 1 of 11 experimental diets from 0 to 21 d post-hatch. A corn and soybean meal-based basal diet was formulated to contain 0.20% available P (0.47% total P), 0.70% total Ca, and no phytase. This basal and OP and OPP summit diets (4,000 FTU/kg) of identical composition were pelleted, crumbled, and analyzed for phytase activity and subsequently blended in crumbled form to achieve target phytase activities in experimental diets of 0 (basal), 125, 250, 500, 750, or 1,500 FTU/kg from either OP or OPP. Treatments were administered to 6 replicate cages of 8 chicks (528 total). The basal diet was analyzed to contain 0.40% and 0.66% total P and Ca, respectively. The analyzed phytase activity of experimental feeds ranged from 41 to 95% and 72 to 105% of target activity for OP and OPP diets, respectively. Data were analyzed by ANOVA as a 2 (phytase source) × 5 (phytase level) factorial plus control (OP; OptiPhos Plus, Huvepharma, Peachtree City, GA) experiment, which consisted of 2 phytase doses (1,000 and 2,500 phytase units/kg; FYT) and 2 SBM with different protein concentration (45 and 47% of CP), totaling 4 dietary treatments with 12 replicates of 25 birds each. The birds received feed and water ad libitum. Diets were based on corn and SBM. The SBM with 48% CP was used as basis for diet production, included at 30% for all diets, and different levels of soybean hulls were added to dilute the protein concentrations of the SBM according to each treatment. These adjustments were possible due to modifications in corn and soybean oil inclusion. At 21d of age, 60 birds per treatment were randomly selected and euthanized, and ileal content was collected. Feed and ileal samples were dried, ground, and analyzed to determine the apparent ileal digestibility (CAID) of dry matter (DM), crude protein (CP), phosphorus (P), calcium (Ca) and apparent digestible energy (ADE). At 21 d, blood samples from 24 broilers per treatments were collected to determine the myo-inositol level (MYO) in the plasma. The data were analyzed using ANOVA and when interactions were detected, means were compared by Tukey test at P<0.05. No interaction (P > 0.05) was observed between factors for CAID variables and plasmatic MYO at 21-d-old. The protein concentration of SBM had a significant effect only on CAID of P (P < 0.05), as the SBM with 45% CP resulted in a 3.92% higher P digestibility compared to SBM 47%. Phytase supplementation had significant effects on CAID of DM and P and also ADE (P < 0.01) which were respectively increased by 2.13%, 8.1%, and 175 kcal with the inclusion of 2500 FYT compared to 1000 FYT. CAID of CP and Ca were neither affected by SBM protein concentration or phytase inclusion (P > 0.05). Increasing phytase dose to 2500 FYT resulted in a significantly greater plasmatic MYO at 21 d than 1000 FYT (P < 0.001). SBM protein concentration had no significant effect (P > 0.05) on

142 Ileal digestibility and myo-inositol plasma concentration of broiler chickens fed high phytase doses and different soybean meal protein content. Leopoldo M. Almeida*, Lucas S. Bassi*, Rafael Sens*, Vitor Augusto B. Zavelinski*, Levy Teixeira*, Alex Maiorka*, Veterinary Science, Federal University of Paraná, Curitiba, Brazil, Department of Animal Sciences, Federal University of Paraná, Curitiba, Paraná, Brazil, DSM Nutritional Products, São Paulo, Brazil.

This study evaluated the ileal nutrient digestibility and plasmatic myo-inositol of broiler chickens fed diets two phytase doses and two soybean meal (SBM) with different protein levels. A total of 1,200 one-d-old broilers were distributed in a completely randomized design using a 2x2 factorial arrangement, which consisted of 2 phytase doses (1,000 and 2,500 phytase units/kg; FYT) and 2 SBM with different protein concentration (45 and 47% of CP), totaling 4 dietary treatments with 12 replicates of 25 birds each. The birds received feed and water ad libitum. Diets were based on corn and SBM. The SBM with 48% CP was used as basis for diet production, included at 30% for all diets, and different levels of soybean hulls were added to dilute the protein concentrations of the SBM according to each treatment. These adjustments were possible due to modifications in corn and soybean oil inclusion. At 21d of age, 60 birds per treatment were randomly selected and euthanized, and ileal content was collected. Feed and ileal samples were dried, ground, and analyzed to determine the coefficient of apparent ileal digestibility (CAID) of dry matter (DM), crude protein (CP), phosphorus (P), calcium (Ca) and apparent digestible energy (ADE). At 21 d, blood samples from 24 broilers per treatments were collected to determine the myo-inositol level (MYO) in the plasma. The data were analyzed using ANOVA and when interactions were detected, means were compared by Tukey test at P<0.05. No interaction (P > 0.05) was observed between factors for CAID variables and plasmatic MYO at 21-d-old. The protein concentration of SBM had a significant effect only on CAID of P (P < 0.05), as the SBM with 45% CP resulted in a 3.92% higher P digestibility compared to SBM 47%. Phytase supplementation had significant effects on CAID of DM and P and also ADE (P < 0.01) which were respectively increased by 2.13%, 8.1%, and 175 kcal with the inclusion of 2500 FYT compared to 1000 FYT. CAID of CP and Ca were neither affected by SBM protein concentration or phytase inclusion (P > 0.05). Increasing phytase dose to 2500 FYT resulted in a significantly greater plasmatic MYO at 21 d than 1000 FYT (P < 0.001). SBM protein concentration had no significant effect (P > 0.05) on
Plasmonic MYO concentration. In conclusion, the highest phytase dose in broiler diets improves the coefficient of apparent ileal digestibility of DM, P, and ADE, in addition to increasing plasma MYO.

Key Words: Enzyme, Phosphorus, Nutrient Digestibility, Myo-inositol, SBM

143 Phytase doses and soybean meal protein content on growth performance, bone mineral composition and toe ash of broilers. Vitor Augusto B. Zavelinski, Lucas S. Bassi, Rafael Sens, Gabriela d. Pilon, Kariny F. da Silva, Levy Teixeira, Simone G. de Oliveira, Federal University of Paraná, Curitiba, Paraná, Brazil, 2Department of Animal Sciences, Federal University of Paraná, Curitiba, Paraná, Brazil, 3DSM Nutritional Products, São Paulo, São Paulo, Brazil.

The objective of this study was to evaluate the effects of different doses of phytase and protein content of two soybean meal (SBM) on growth performance, bone mineral composition and middle toe ash of broilers. A total of 1200 one-d-old Cobb 500® broiler chicks were distributed in a completely randomized design using a 2x2 factorial arrangement from 1 to 21 d. The dietary treatments were iso-nutritious (CP= 18%; Ca= 0.78%; P= 0.39%; Lys= 0.98%; Met= 0.49% and Met +Cys=0.74%) with 2 doses of phytase (1000 and 2500 phytase units/kg; FYT) and 2 protein content levels of soybean meal (45 and 47% crude protein) totaling 4 treatments with 12 replicates of 25 birds each. Feed intake, body weight gain and feed conversion ratio in the period were determined. At 21 d, 24 broilers per treatment were euthanized for tibia bone collection to determine ash, Ca, and P content, and Seedor Index (bone weight divided by its length). The middle toe was also collected to evaluate toe ash. The data was tested for residue normality by the Shapiro-Wilk test, and after a normal distribution was detected, data was submitted to ANOVA. When significant interactions were observed, their deployment was submitted to Tukey test at 5% probability for mean comparison. No interaction was observed between factors (P<0.05) on growth performance variables from 0–21 d. Separately, the inclusion of 2500 FYT/kg increased body weight gain in 3.11% (maximum of 808.43 g; P<0.05) compared with the diet containing 1000 FYT/kg, whereas the content of protein of SBM had no significant effect on growth performance (P>0.05). An interaction (P<0.05) was detected for Ca content in the tibia bone, which was lower for broilers fed the diet containing 1000 FYT/kg and SBM with 45% of protein (19.8%), whereas the other treatments had statistically equal results for bone Ca (to a maximum of 22.82%). Diets with 2500 FYT/kg resulted in greater total bone ash (58.4%), Seedor Index (27.53) and toe ash (44.13%) compared to the lower dose (56.3%, 25.52, and 42.44%, respectively; P<0.01), but P content in the bone did not differ (P>0.05). In conclusion, the supplementation of broiler chicken diets with higher levels of phytase up to 2500 FYT increased bone mineral density and ash content of bone and toe, and also improved weight gain from 1-21 d, while the protein content of SBM did not influence growth performance and mineral bone composition.

Key Words: Ash, Growth Performance, Mineral Composition, Phytase, Soybean Meal

144 Comparative efficacy and dose effects of a novel consensus bacterial 6-phytase variant and a commercial 6-phytase on retention of phosphorus and calcium in egg laying hens fed inorganic P-free diet. Abiodun Bello, Mick Roberts, Yueming Derjant-Li, Leon Marchal, DuPont Nutrition & Biosciences, Oegstgeest, South Holland, Netherlands, 2AH Pharma, Inc, Salisbury, Maryland, United States.

Phytase is typically used in laying hen diets at approximately 300 FTU/kg, the need for improved nutrient efficiency and a more sustainable laying hen industry necessitates the drive for an increased dose of a highly bio-efficient phytase. The dose effect of a novel consensus bacterial 6-phytase variant expressed in Trichoderma reesei (PhyG) and its comparative efficacy relative to a Buttiauxella sp. 6-phytase also expressed in Trichoderma reesei (PhyB) were assessed based on apparent total tract digestibility (retention) of Ca and P in egg laying hen. A total of 216 Lohmann LSL Lite hens (3 birds × 72 cage) were fed common nutrient adequate pre-experimental diet through 24 weeks of age (woa) and were fed one of 6 experimental diets from 25-29 woa. The diets were corn-soybean meal-rice bran (0.33% phytate) based and were fed ad libitum. The diets were a nutrient adequate positive control (PC), a nutrient reduced NC (the PC without inorganic P / bone meal and reduced in Ca by 0.334% unit) and the NC supplemented with the PhyG at 300 and 600 FTU/kg or with the PhyB at 300 and 600 FTU/kg. The phytase analysis in the PhyG-supplemented diets were 281 and 496 FTU/kg and in the PhyB-supplemented diets were 286 and 617 FTU/kg, respectively for the targeted 300 and 600 FTU/kg doses. Excreta samples were collected in the last 4 days of the trial for determination of P and Ca retention. Data analysis was done using one-way ANOVA of JMP 14. The P retention was decreased for NC (27.25%) vs PC (31.71%) and was increased at the 300 FTU/kg dose by 8.33 and 8.18% units and at the 600 FTU/kg dose by 10.83 and 10.77% units for the PhyG and PhyB vs NC, respectively (P<0.001). However, Ca retention was not different between PC (64.33%) and NC (61.07%), while the parameter was increased (P<0.05) at the 300 FTU/kg dose only by the PhyG (+6.12%, unit), and at the 600 FTU/kg dose by both PhyG (+8.97%, unit) and PhyB (+7.63%, unit) vs NC. At each of 300 and 600 FTU/kg doses, the novel consensus bacterial 6-phytase variant had consistently higher Ca retention than the commercial 6-phytase. Overall, the result indicated that both phytases improved P and Ca retention, however the novel consensus bacterial 6-phytase variant demonstrated the highest Ca retention-increasing efficiency at same phytase dose and increasing the phytase dose from 300 to 600 FTU/kg significantly improve P and Ca retention in egg-laying hens.
Key Words: laying hens, inorganic phosphorus-free, phosphorus, digestibility, phytase


This study evaluated the efficacy of a novel consensus bacterial-6 phytase variant expressed in T. reesei (PhyG) in broilers fed diets reduced in nutrients based on three doses of phytase, respectively. A total of 2,016 straight run Ross 708 broilers (24 birds x 12 floor pens per treatment) were randomly allocated to each of 7 dietary treatments. The positive control (PC) was formulated to meet nutritional requirements. Negative controls 1, 2, and 3 (NC1, NC2, and NC3) ranged in 0.21–0.24% of phytase P were the PC respectively reduced in ME by 62.8, 68.8, and 69.5 kcal/kg, available P (ap) by 0.15%, 0.18%, and 0.19%, Ca by 0.17%, 0.20%, and 0.21%, dig AA by 0.02-0.05% and Na by 0.03-0.05%. Other diets were the NC1, NC2, and NC3 respectively supplemented with PhyG at 500 (PhyG500), 1000 (PhyG1000), or 2000 (PhyG2000) FTU/kg. Diets were based on corn/SBM with up to 2.5% meat and bone meal and were fed ad libitum over four phases; starter (d1-10), grower (d11-21), finisher 1 (d22-35), and finisher 2 (d36-48). Feed intake and bodyweight were measured at d1, 10, 21, 35, and 48 to calculate body weight gain (BWG), feed consumption (FC), and BW-corrected feed conversion ratio (FCRc). At d21, four birds per pen were sampled for ileal digesta contents collection for the determination of apparent ileal digestibility (AID) of Ca and P and for right tibia bone collection for determination of bone ash. Feed cost/BWG, USD/kg was also estimated for the 0-48d period. All data was analyzed via One-way ANOVA using the GLM model. Treatment means were further separated using Tukey’s HSD test. Compared to the PC (3,470 g), d48 BW decreased (P<0.05) by NC2 (3,248 g) and NC3 (3,195 g) with complete alleviation by PhyG1000 (3,444 g) and PhyG2000 (3,449 g), respectively. The d0-48 FCRc showed similar response as d48 BW, NC1, NC2 and NC3 increased FCRc by 6, 10 and 13 points, PhyG at all dose levels maintained FCRc vs PC (P>0.05). Each of the 3 NC diets decreased (P<0.05) tibia ash and AID of Ca and P vs PC, and each of PhyG500, PhyG1000, and PhyG2000 completely restored tibia ash and AID of Ca and P to same levels as the PC. While the application of respective matrix values in the corn/SBM diets across the four phases lowered feed cost by 9.7, 11.6 and 12.7 USD/ton at phytase dose of 500, 1,000 and 2,000 FTU/kg, respectively, the d0-48 feed cost / BWG, USD/kg was lowered by PhyG500 (0.439), PhyG1000 (0.432), and PhyG2000 (0.433) vs PC (0.454). In conclusion, the novel consensus phytase variant completely compensated for the reduction in dietary aP, Ca, dig AA, and ME at each dose level and maintained growth performance and bone quality of broilers through 48 days of age, leading to reduced feed cost and production benefit.

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

146 Comparative efficacy of a novel consensus bacterial 6-phytase variant and a commercial-6 phytase at 2,000 FTU/kg on growth performance, tibia ash and economic benefit in broilers. Abiodun Bello⁎*, Eric B. Sobotik⁎, Gregory S. Archer*, Yueming Derjant-Li*, Leon Marchal*, DuPont Nutrition & Biosciences, Oegstgeest, South Holland, Netherlands, Poultry Science, Texas A&M University, College Station, Texas, United States.

The comparative efficacy of a novel consensus bacterial-6 phytase variant expressed in Trichoderma reesei (PhyG) and a commercial hybrid 6-phytase expressed in Aspergillus niger (PhyH) at 2,000 FTU/kg dose was evaluated. Cobb 500 male broilers (a total of 960: 20 birds x 12 floor pen replicates x 4 dietary treatments) were fed one of the 4 diets over 4 phases (0-10, 10-21, 21-35, and 35-42d). Two controls included were a nutrient adequate PC and a nutrient reduced NC with 0.33 phytate P (the PC minus 87.8 kcal/kg ME, 0.72-1.03% crude protein, 0.064-0.084% dig Lys, 0.199% available P, 0.21% Ca, and 0.047% Na). Other diets were the NC supplemented with 2,000 FTU/kg of PhyG (PhyG2000) or PhyH (PhyH2000). The phytase analysis averaged across the 4 phases were 2,213 (PhyG2000); and 2,931 (PhyH2000) FTU/kg. The diets were based on corn, soybean, wheat, rapeseed meal, rice bran, and oat hulls. Growth and feed efficiency performance parameters were collected per phase and on the cumulative 0-42d. Day 0-42 FCR was corrected for BW (FCRc). On 21d, tibia bone samples were collected from 2 birds per pen for bone ash content. Economic analysis was performed based on the cost of feed needed per kg of BWG. One-way ANOVA was employed for statistical analysis of data using JMP 14 and Tukey test for treatment mean separation. Day 42 BW was decreased (P<0.05) by NC (2,772 g) vs PC (3,087 g); and PhyG2000 (3,125 g) improved (P<0.05) BW vs NC to the same level as PC, while PhyH2000 (3,019 g) was intermediate between the controls. Diet effects on majority of the growth performance parameters at each phase were similar to that of the 42d BW. The 0-42d FCRc was poorer (P<0.05) for the NC (1.799) vs PC (1.646), however, only PhyG2000 (1.666) improved (P<0.05) FCRc vs NC, while PhyH2000 (1.709) was intermediate to the controls. The 21d tibia ash was also reduced (P<0.05) by the NC vs PC and while it was completely restored (P<0.05) to the same level as the PC by the PhyG2000, tibia ash was increased vs NC but decreased vs PC by the PhyH2000 (P<0.05). Feed cost per kg BWG was lower for PhyG2000 at 0.473 USD/kg BWG and PhyH2000 at 0.483 USD/kg BWG vs that of the PC at 0.501 USD/kg BWG. Overall, the result showed that the 2,000 FTU/kg of the PhyH only partially compensated for the nutrient reduced and the 2,000 FTU/kg of the novel
consensus bacterial 6-phytase variant more cost-effectively compensated for the energy and nutrient downspec to maintain tibia ash and performance comparable to PC.

Key Words: broiler, bone ash, growth performance, novel phytase, phytase dose


This study evaluated the effect of a novel consensus bacterial 6-phytase variant (PhyG) on phosphorus (P) retention, tibia ash and performance in broilers fed mash or pelleted diets. A total of 108, day-old Ross 308 male broilers were fed a common diet containing an adequate level of nutrients from 1-10 days. On day 10, chickens were weighed and assigned based on body weight (BW) block to six treatments and fed the test diets until day 21 (2 birds/cage, 9 replications/treatment). The 6 treatments were tested in a 2 × 3 factorial arrangement, consisting of two feed forms (mash or pellets) and three diets. The diets included a positive control (PC) containing 0.42% non-phytic P (NPP) and 0.90% Ca, a negative control (NC) without inorganic P containing 0.16% NPP with reduced Ca (0.65%), and NC supplemented with PhyG at 500 FTU/kg. Diets were based on wheat, barley, corn, soybean meal and rapeseed meal containing 0.16% NPP with reduced Ca (0.65%), and NC supplemented with PhyG at 500 FTU/kg. Diets were based on wheat, barley, corn, soybean meal and rapeseed meal (analyzed phytate P at 0.31%) and were fed ad libitum. Performance parameters (average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR)) were determined for the 10 to 21 days period. On day 21, the right tibia bone from 2 birds per cage were collected and pooled, for the measurement of whole tibia ash and P content in ash. Excreta samples were collected for 5 days (16 to 21 days) to determine P retention. The data was analyzed by 2-way ANOVA (SAS) and treatment mean separation by Tukey’s multiple range test. No interaction between feed form and diets was observed for any of the performance parameters. The NC reduced (P<0.05) ADG, ADFI and increased FCR vs. PC, PhyG at 500 FTU/kg completely recovered all performance parameters compared with PC, regardless of feed form. A feed form × diet interaction (P<0.001) was found for tibia ash: in mash, it was reduced by 11% point for NC vs. PC; while in pellet diets, it was reduced by 6.1% point for NC vs. PC and tibia ash content was completely recovered by PhyG vs. PC, in both feed forms. Feed form did not have an impact on P retention. Across feed forms, PhyG at 500 FTU/kg increased P retention by 22.7% points vs. NC and maintained retainable P (g/kg) comparable to PC. In conclusion, birds fed pelleted diets showed greater ADFI, ADG, FCR, tibia ash and P content in ash compared to those fed mash form. However, the novel consensus phytase variant at 500 FTU/kg improved P retention and growth performance compared to NC (without supplementation of inorganic P), regardless of feed form, restored performance, bone ash and retainable P to the level of PC.

Key Words: consensus phytase variant, broilers, performance, P retention, tibia ash

148 A novel consensus bacterial 6-phytase variant increased phosphorus and calcium retention and productivity parameters in egg laying hens fed inorganic P-free diet. Arun Kumar*,1, Abiodun Bello2, Yueming Derjant-Li1, ‘School of Agriculture and Food Science, University of Queensland, Gatton, Queensland, Australia, 2Nutrition & Biosciences, DuPont, Oegstgeest, South Holland, Netherlands, 3Animal Nutrition, DuPont Nutrition and Biosciences, Oegstgeest, Netherlands.

The efficacy of a novel consensus bacterial 6-phytase variant (PhyG) to maintain lay rate, eggshell quality, and nutrient digestibility of laying hens fed inorganic P-free diet was evaluated. A total of 384, 16 weeks old, ISA brown layers were randomly allocated to two dietary treatments each with 48 cage replications of four hens per cage in an experimental layer house. All hens were fed common layer mash diet from 16 to 23 weeks of age (woa). The experimental diets were corn, soybean, canola meal, and rice bran-based (0.282% phytate P) and were adequate in energy and all other nutrients (based on breeder recommendation) except for available phosphorus (avP) and calcium (Ca). With no added inorganic P or bone meal in diets, avP and Ca levels in the experimental diets were at 0.127 and 3.276%, respectively, hence reduced by 64 and 10% vs the respective recommended levels. The control diet without or with PhyG at 300 FTU/kg, were fed ad libitum from 24 to 47 woa. Egg production performance and egg quality parameters were measured during the 23 weeks experimental period. Retention of P and Ca were measured in excreta samples collected within the first 7-10 days of study using 2 methods, the acid insoluble ash (2% Celite) and the total collection method. Data were analyzed using one-way ANOVA with SAS 9.4. Relative to the average egg production (HDEP) of 95% expected for ISA Brown hens across 24-47 woa, egg production was decreased to 91.8% by the control diet. Supplementation of 300 FTU/kg PhyG in inorganic P free layer diet significantly (P<0.05) improved egg production to 93.7% and egg mass (53.4 vs 54.6 g), and feed intake (113.6 vs 116.3 g) without affecting FCR. Based on the acid insoluble ash method, PhyG supplementation at 300 FTU/kg increased (P<0.05) P retention (19.2 vs 27.9%) and Ca (64.1 vs 66.1%) and based on total collection method, PhyG at 300 FTU/kg increased (P<0.05) P retention (20.5 vs 33.4%) while there was no diet effect on Ca retention. Egg quality parameters (egg breaking strength and shell thickness) assessed at 20 and 23 weeks of study were also improved (P<0.05) by PhyG supplementation. Overall, the data demonstrated the efficacy of the PhyG at 300 FTU/kg to efficiently liberate P for improvement of 1.9% egg production across 24-47 woa in hens fed inorganic P or bone meal-free diet for 23 weeks.
In conclusion, the novel consensus bacterial phytase variant increased P and Ca retention and egg productivity compared to the control, resulting in decrease of P excretion to the environment.

Keywords: laying hens, inorganic phosphorus-free, egg production, nutrient retention, and phytase

Key Words: laying hens, inorganic phosphorus-free, egg production, nutrient retention, phytase

149 Effect of a novel consensus bacterial 6-phytase variant on apparent ileal digestibility of amino acids in broilers at 15 days of age. Yueming Derjant-Li*1, M. Reza Abdollahi2, Katie Waller1, Abiodun Bello1, Leon Marchal1, V. Ravindran2, 1Animal Nutrition, DuPont Nutrition and Biosciences, Oegstgeest, Netherlands, 2School of Agriculture and Environment, Massey University, Palmerston North, New Zealand.

This study evaluated the effect of a novel consensus bacterial 6-phytase variant (PhyG) on apparent ileal digestibility (AID) of amino acids (AA), phosphorus (P) retention and bone ash in broilers. A total of 256 male 5-d-old Ross 308 broiler chicks were allocated to 4 treatments (8 replicate cages and 8 birds/cage). The 4 diets included: 1) a positive control (PC, 2,975 kcal/kg ME, 1.06% dig Lys, 20.4% crude protein, 0.37% dig P, 0.84% Ca, and 0.18% Na); 2) a negative control (NC) without inorganic P, with reduced ME (68 kcal/kg), dig AA (0.03-0.04% unit), Ca (0.20% unit), dig P (0.22% unit) and Na (0.04% unit) vs. PC; 3) and 4): the NC supplemented with the PhyG at 500 and 1,000 FTU/kg, respectively. The analyzed phytase activity was 541 and 991 FTU/kg (above NC), respectively. Diets were corn/soy/rapeseed-meal/rice-bran based with 0.5% titanium dioxide and were fed in mash form from 6-15 d of age. After 6 d of adaptation, excreta samples were collected for 4 d to determine the P retention. On d 15, digesta from the distal ileum was collected to determine AID of AA, DM, N and GE. The digestible AA content in the diet (dig AA, %) was calculated for individual AA and the total digest AA (n=17), based on AID and the analyzed AA content of diets. Tibia ash was measured in a pooled sample of 6 birds per cage collected at d 15. Data were analyzed by one-way ANOVA (JMP 14.1). Tukey’s test was used for multiple treatment mean comparisons and LSMeans contrast for pair comparisons. The NC decreased (P<0.05) all individual dig AA (n=17) vs PC. Increasing phytase dose linearly increased (P<0.05) the AID and dig of all 17 individual AA and total AA. At 1,000 FTU/kg, PhyG added to dig AA-deficient NC diets improved (P<0.05) dig AA content for all 17 individual AA vs. NC. The PhyG improved the dig AA in a range of 3.2% (Met) to 11.3% (Cys), with an average of 5.9% at 500 FTU/kg and 3.7% (Met) to 14.4% (Cys), with an average of 7.5% at 1,000 FTU/kg vs. NC. Based on contrast analysis, PhyG increased the AID of DM, N and GE by 4.7, 5.1 and 4.5% points, respectively, vs. NC (P<0.05). Tibia ash was reduced (P<0.05) in NC (28.3%) vs. PC (42.6%) and PhyG at both dose levels improved (P<0.05) the tibia ash vs. NC. PhyG at 1,000 FTU/kg was able to fully recover tibia ash content (42.1%) vs. PC (P>0.05). The P retention was improved (P<0.05) by 16 and 25% points by PhyG at 500 (63.1%) and 1,000 FTU/kg (71.6%) as compared to the NC (46.7%). In conclusion, the novel consensus bacterial 6-phytase variant efficiently improved AID of DM, N, GE and AA, P retention and tibia ash in broilers, demonstrating both phosphoric and extra-phosphoric effects

Key Words: consensus phytase variant, broiler, digestible amino acids, digestibility

150 Effects of phytase supplementation and nutrient uplifts on growth performance, meat yield, hypothalamic gene expression, and catecholamine concentrations in broilers from 1 to 43 days of age. Ruben Kriseldi*1, Laci MacKay2, Chad Foradori1, Ryan Dilger1, Mike Bedford2, Carrie L. Walk3, William A. Dozier1, 1Poultry Science, Auburn University, Auburn, Alabama, United States, 2AB Vista, Marlborough, United Kingdom, 1Animal Science, University of Illinois, Urbana, Illinois, United States, ‘Anatomy, Physiology, and Pharmacology, Auburn University, Auburn, Alabama, United States.

Two experiments were conducted to evaluate extra-phosphoric effects of phytase on growth performance, feed intake, meat yield, hypothalamic gene expression of appetite hormones, and dopamine and serotonin concentrations of broilers. Experiment 1 was designed to determine differences of digestible amino acid concentration and AMEa using 256 Yield Plus × Ross 708 broilers (32 cages, 8 birds/cage) that were fed diets with or without phytase supplementation at 4,500 phytase units (FTU/kg (16 reps/treatment). These differences were used as nutrient uplifts in Experiment 2 to mimic effects of phytase on increasing nutrient intake. In Experiment 2, 832 Yield Plus × Ross 708 broilers were placed in 32 floor pens (26 birds/pen). Diets were provided in a 2 × 2 factorial arrangement consisting of 2 nutrient contents (basal or uplifts) and 2 phytase inclusions (0 or 4,500 FTU/kg). Diets with nutrient uplifts were formulated to contain 0.007, 0.015, 0.013, 0.021, 0.024, and 61 kcal/kg higher than the lowest (0.01) carcass and breast meat weights among dietary treatments. At 43 d of age, blood and hypothalamus were collected from 4 birds/pen to determine plasma inositol and hypothalamic gene expression and concentrations of appetite hormones. Data were analyzed using 1-way (Experiment 1) or 2-way (Experiment 2) ANOVA. In Experiment 2, broilers fed diets formulated with nutrient uplifts and phytase addition from 1 to 40 d had the lowest (P<0.05) feed conversion and the heaviest (P<0.01) carcass and breast meat weights among dietary treatments. Nutrient uplifts resulted in a 97 g increase (P<0.05) in BW gain of broilers compared with birds fed diets without nutrient uplifts from 1 to 40 d of age. Effects of

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An experiment was conducted to evaluate the effects of high levels of phytase on nutrient apparent ileal digestibility and myo-inositol (MYO) concentration in the plasma of turkeys. A total of 320 one-d-old male turkeys were distributed in a completely randomized design with 4 treatments and 8 replicates of 10 birds each. Feed was offered ad libitum in mash form, based on corn and soybean meal. The dietary treatments were: positive control diet (PC); negative control diet (NC, with reduction of 0.15% available P and 0.18% Ca); NC + 2000 FYT/kg; NC + 4000 FYT/kg. At 28 d-old, two birds of each replicate were randomly chosen and euthanized to collect ileal content samples, which were analyzed for ileal digestible energy (IDE). On d 28, blood samples were collected from two turkeys per replicate and analyzed for plasmatic MYO (IDE). On d 28, blood samples were collected from two turkeys per replicate and analyzed for plasmatic MYO concentration. Data were submitted to ANOVA and means compared with Tukey test at 5% probability. A linear regression analysis was evaluated between NC diet and the two phytase doses. Phytase supplemented diets resulted in greater P digestibility (P < 0.05) compared to control diets, and the effect of increasing the phytase dose on P digestibility was linear (P < 0.05), with a maximum of 75.6%. A linear trend (P < 0.1) of increasing phytase dose was observed for crude protein digestibility. MYO concentration in the plasma was higher on turkeys fed phytase supplemented diets (P < 0.05), and increasing the dose had a linear effect on MYO (P < 0.05). In conclusion, the supplementation of low Ca and P turkey diets with high levels of phytase up to 4000 FYT/kg linearly improves P ileal digestibility and MYO absorption.

Key Words: broiler, phytase, inositol, dopamine, serotonin

152 Effect of phytase type and inclusion level on broiler performance and tibia ash from 1 to 21 d of age.


Around 70% of phosphorus (P) in plant feedstuffs is present in a phytate form and biologically unavailable to poultry. Phytate binds to minerals and other nutrients reducing their availability and absorption. Exogenous phytases are commonly used in poultry diets to increase P availability, reduce feed costs and P excretion. The objective of this study was to evaluate the effects of phytase type (coated-OptiPhos (OP) and uncoated-OptiPhos+ (OP+), an intrinsically heat stable form of OptiPhos) and inclusion level on broiler performance and tibia ash from 1 to 21 d of age. Treatments consisted of a positive control (PC) formulated to contain 1.00% Ca and 0.40% avP, a negative control (NC) formulated to contain 0.80% Ca and 0.20% avP, and the NC + 250, 500, 1000 or 1500 FTU kg-1 of either OP or OP+. A total of 640 d-old male broilers were randomly distributed in battery cages among 10 treatments with 8 replicate cages per treatment. Body weight (BW) and feed intake (FI) were determined at 7, 14, and 21 d of age and FCR was corrected for mortality. On d 21, 3 birds/cage were euthanized, and left tibias were collected to analyze bone breaking strength, using an Instron texture analyzer, and tibia ash. Data were analyzed using JMP 14, as a completely randomized block design in a 2×4 factorial arrangement to identify main effects and interactions of phytase type and inclusion level, as well as PC and NC. Broilers fed the NC had lower (P<0.05) BW and FI than broilers fed the PC and OP+ diets. While broilers fed the NC had similar (P>0.05) BW, FI and FCR to broilers fed OP diets. Broilers fed the PC had higher (P<0.05) BW (953 vs. 889g), FI (1079 vs. 1017g) and lower FCR (1.21 vs. 1.23g:1) than broilers fed OP diets. Broilers fed with OP had higher (P<0.05) BW (942 vs. 889g), FI (1060 vs. 1017g) and lower FCR (1.20 vs. 1.23g:1) than broilers fed OP+, but similar (P>0.05) to broilers fed the PC. Enzyme inclusion level didn’t influence BW and FCR at 21 days of age. However, broilers fed diets with 250 and 1000 FTU, had lower (P<0.05) FI than broilers fed diets with 1500 FTU. The addition of either OP or OP+ increased tibia ash and bone breaking strength of broilers when diets were deficient in avP. Broilers fed diets with OP had higher (P<0.05) tibia ash (36.02 vs. 34.22%) and bone breaking strength (33.7 vs. 29.9N) than broilers fed OP diets. Broilers fed diets with 250 FTU had the lowest (P<0.05) tibia ash of all phytase inclusion levels. In addition, bone breaking strength was the highest (P<0.05) in broilers fed diets with 1000 and 1500 FTU. These results indicate that phytase type and inclusion level influence broiler performance, bone breaking strength, and tibia ash from 1 to 21 d of age.
Key Words: coated, phosphorus, phytase, performance, tibia ash

153 Total inorganic phosphorus free and more economical broiler diets supplemented with a novel consensus bacterial 6-phytase variant maintained normal growth characteristics in all phases. Leon Marchal1*,2, Yueming Derjant-Li1, Abiodun Bello1, Eric B. Sobottik4, Gregory S. Archer4, DuPont Animal Nutrition, Warnsveild, Netherlands, 'Animal Nutrition, Wageningen University, Wageningen, Netherlands, 'Nutrition & Biosciences, DuPont, Oegstgeest, South Holland, Netherlands, ‘Poultry Science, Texas A&M University, College Station, Texas, United States.

There is a global growing sustainability awareness on the proper use of finite resources like inorganic phosphorus (P) and reduction of pollution. While broiler meat production has one of the lowest environmental impact of all animal proteins, in commercial practice it still uses significant amounts of inorganic phosphorus. This study investigated if it would be possible to have a total inorganic P free, practical relevant broiler diet with normal growth characteristics in all growth phases. A nutrient adequate, but not over-specified, 4 phased (0-10, 10-21, 21-35, 35-42 days) mixed diet with corn, wheat, soybean meal, rapeseed meal, rice and wheat bran was used to provide enough substrate (i.e. phytate). Oat hulls were also added to provide enough structure in the diet, and calcium levels were relatively low for the different phases. Two separate trials were conducted to increase the robustness of the findings. Data from the two trials were analyzed together (trial as random effect). Each trial contained 6 treatments, with 10 replicates per treatment (Ross 308; 24 birds/pen, 50% male & 50% female in trial 1; 26 birds/pen, 100% male in trial 2). Two different positive controls (PC) with mono-calcium phosphate (MCP) and without phytase were used (PC1), one with xylanase added (PC2). The 4 test groups were fed diets without MCP, with a different dosing strategy for a novel consensus bacterial 6-phytase variant (i.e. 1000, 2000 or 3000 FYT/kg in the starter phase) and one treatment also had a xylanase added (2000 XU/kg xylanase plus 2000 FYT/kg phytase in starter phase). Data from the two trials were analyzed together by one-way ANOVA (trial as random effect) using JMP 14, treatment means were separated by Tukey test (P<0.05 as significant). All treatments had comparable or better growth and feed conversion vs. the breeder performance objective for Ross 308. The treatment with phytase added at 3000 (starter), 2000 (grower), 1000 (finisher 1&2) FTU/kg improved (P<0.05) body weight and feed conversion compared to the PC1 during 0-21 and 0-42 days of age. All test groups showed greater (P<0.05) breast yield compared to PC1. Bone breaking strength of the inorganic P free diets did not deteriorate compared to the controls, on the contrary at 42 days the bone strength was significantly higher (P<0.05) for the highest starter phytase dose (3000 FYT) and 2000 FYT phytase + xylanase. The feedcosts per kg body weight gain were 2-5% lower compared to PC1. To the best of our knowledge this is the first report to show a 100% vegetable total inorganic P free commercial relevant broiler diet with normal growth characteristics in all growth phases.

Key Words: inorganic phosphor free, consensus 6-phytase, sustainability, broiler

154 In vitro set up to compare Phosphate (PO43-) availability in feed diet formulations. Diogo F. Rosso*,1, Jacqueline C. de Souza1, Levy Teixeira1, Rafael Sens1, Lucas S. Bassi1, Alex Maiorka2, Novozymes Latin America, Araucária, Parana, Brazil, 2Department of Animal Sciences, Federal University of Paraná, Curitiba, Paraná, Brazil, 3DSM Nutritional Products, Maîrinque, Sao Paulo, Brazil.

The objective of this study was to evaluate the effect of different phytase doses on phosphate groups release after in vitro incubation of feed diet formulations. Feed formulations included two doses of phytase (1000 and 2500 phytase units/kg of feed, FYT) and two soybean meal (SBM) with different protein content (45% and 47% of crude protein adjusted with corresponding amounts of soybean hull). Feed was formulated for four different phases, from 1-7, 8-21, 22-35 and 36-42 days. For the in vitro incubation model, formulated diet was incubated at 15% of dry matter (DM) and pH 3.00±0.25 for 15 minutes, followed by pH 6.5±0.25 at 37°C for 4 hours to simulate crop and intestinal digestion phases, respectively. Supernatants were collected once incubation time was finalized, filtered and further diluted for quantification of released phosphate by high performance anion-exchange chromatography with conductivity detection (HPAEC-CD). Standard solution series with phosphate in 6 concentration levels (0.0025-0.075 g/l) were used for quantification, which was performed on an ICS-5000 (Dionex, Thermo Scientific) equipped with an IonPac AS11-HC column (250 x 4 mm, Dionex) and an IonPac AS11-HC guard-column (4 x 50 mm, Dionex). 30mM potassium hydroxide (KOH) mobile phase was used with a 1.5ml/min flow. Soluble phosphate released upon phytase action on the different feed materials were submitted to One-way ANOVA test. The inclusion of 2500 FYT/kg led to a statistically significant increase (8.01%) on phosphate release over 1000 FYT/kg considering all diets data combined, whereas no significant effect was observed from the two protein contents of SBM tested. It was observed, however, a higher effect of 2500 FYT/kg over 1000 FYT/kg for the diet with 47% protein SBM, in which an increase of 11.94% was determined, while for the diet with 45% protein SBM a non-significant increase of 4.26% was verified. In conclusion, in vitro incubation set-up and anion-exchange chromatography were successfully employed to evaluate the effect of phytase dosages on formulated feed diets by analyzing phosphate release, with significant differences observed between two phytase doses. The increase on phosphate release by adding 2500 FYT/kg compared to 1000 FYT/kg indicates that the higher phytase dose may bring more
phosphate groups available for the animal and therefore increase phosphorous availability.

**Key Words:** phytase, phosphate, soybean meal, anion-exchange chromatography, in-vitro

155 *Xylanase and protease supplementation to diets with varying levels of fiber and protein influenced the jejunal digesta hexose and pentose oligosaccharides profiles and nutrient utilization in broiler chickens.* Yang Lin**, Oluyinka Olukosi, Poultry Science, University of Georgia, Athens, Georgia, United States.

A total of 384 Cobb 500 male broiler chicks were used in a 21-day experiment to study xylanase and protease influence on digesta oligosaccharides profile and nutrient utilization responses in broilers receiving different dietary levels of fiber (F) and protein (P). A total of 16 diets arranged in 2×2×2×2 factorial for effects of fiber (low, L or high, H), protein (L or H), xylanase (without or with), and protease (without or with) were used. The 16 diets consisted of four basal diets: LFHP, LFLP, HFHP and HFLP supplemented with xylanase or protease individually or in combination. Wheat bran was used to increase the dietary fiber content and the HF diets had lower ME. All the diets were supplemented with phytase at 500 FTU/kg. Birds were allocated to the 16 dietary treatments in a randomized complete block design on day 0 of age. Each treatment had 6 replicates with 4 birds per cage. Jejunal, ileal digesta and excreta were collected on d 21. The concentration of oligosaccharides in jejunal digesta was analyzed by matrix-assisted laser desorption ionization mass spectrometry analysis. The data were analyzed by the mixed model procedure of JMP Pro 14 for a randomized complete block design and a factorial treatment arrangement. There were no 4-way interactions for jejunal oligosaccharides (pentoses, Pent; and hexoses, Hex) level response. Birds fed HF diet had higher (P < 0.05) digesta concentrations of (Hex)5, (Pent)3 and (Pent)4. There was no interaction of fiber × xylanase × protease interaction explained by the digesta concentrations of (Pent)5 and (Pent)6, whereas enzyme supplementation had no effect. There was significant (P < 0.05) fiber × xylanase × protease interaction explained by the digesta concentrations of (Pent)5 and (Pent)6 being greatest (P < 0.05) in protease-only-supplemented HF diets whereas in the LF diets, combination of xylanase and protease produced greatest (Pent)5 and (Pent)6. There was no significant 4-way interaction for any digestibility parameter measured. There was significant (P < 0.01) fiber × xylanase × protease interaction for ileal DM and nitrogen digestibility. There was significant (P < 0.01 protein × xylanase × protease interaction for AMEn. The interaction is explained by lack of enzyme effect on AMEn in birds fed LP diets. In contrast, in birds fed HP diets, individual enzyme supplementation decreased AMEn (P < 0.05) whereas xylanase and protease combination increased (P < 0.05) dietary AMEn. In conclusion, of all the factors investigated, dietary fiber level had the greatest effect on increasing digesta concentration of pentose and hexose oligosaccharides whereas the enzyme effects are less pronounced due to overlapping interactions. Additional studies are ongoing to further characterize the effect of the enzymes on oligosaccharides profile along the digesta tract of broilers.

**Key Words:** enzyme, xylanase, protease, broiler, oligosaccharides


Although it contains a significant amount of non-starch polysaccharides (NSP), canola meal (CM) is a valuable source of protein for poultry diets. Supplementation of poultry diets containing CM with enzymes is an effective approach to improve the quality of CM. The enzymatic degradation of NSP in diets containing CM will contribute to the production of NSP hydrolysis products, which may have a prebiotic effect in improving animal health and growth. A series of in vitro incubation studies was carried out to determine if various carbohydrase preparations can target NSP of CM. The most effective enzyme combination was studied further in a 3-wk (1-21 d of age) growth performance and NSP digestibility trial with Ross 308 broiler chickens. Broilers were assigned to 3 dietary treatments, each consisting of 8 pens of 5 birds each, and were fed a control corn/CM-based starter and grower diets (Control) or diets supplemented with an enzyme blend at two levels: 0.03 and 0.15%. Starter (1-10d) and grower (11-21d) diets contained 27 and 29% of CM, respectively. All statistical analyses were conducted with SAS (version 9.4, SAS Institute Inc., Cary, NC). Means were separated by Tukey’s honestly significant difference test. In the in vitro studies, the highest NSP depolymerisation was observed for a combination of two pectinase-like enzymes fortified with xylanase, glucanase and cellulase. No further improvement with other enzyme preparations was observed. In comparison to the Control diets, birds fed the diet supplemented with the high concentration of carbohydrases showed significantly (P<0.05) higher body weight gain during the grower phase and the entire trial. Carbohydrase enzyme combination had a significant effect on NSP digestibility (P<0.05), which increased from 0.1% for the Control diet to 19.4%, and 18.6% for the two enzyme-supplemented diets, respectively. Our research suggests that administration of carbohydrase enzyme combination in diets with high inclusion of CM can improve growth performance and NSP digestibility in broiler chickens.

**Key Words:** canola meal, non-starch polysaccharides, NSP, carbohydrase, broiler chicken

157 Effects of an algae-clay complex with or without endogenous enzyme supplementation on the feed

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Several types of feed additives are available to improve feed digestibility. Recently, an innovative algae-clay complex (ACC) developed by Olmix Group (France) has shown to increase the activity of endogenous digestive enzymes and feed efficiency (Gallissot et al., 2019). 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 at the University of Viçosa Poultry Research Center (Brazil). All studies used Cobb 500 male broilers fed corn-soybean diets following the recommendations of Rostagno et al. (2017). Broilers were placed in pens of 20 (Study 1) or 22 (Studies 2 and 3), 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: none was 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. A significance level of <0.05 was considered. Results of Study 1 showed a decrease in feed conversion ratio (FCR) when using the ACC in grower phase (day 22-35; -4%) and total cycle (day 0-35, -3%). Growth rate tended to be higher in the ACC group than control in the grower phase (+4%, P<0.10) with a final weight (at 35 days) of 2.449 kg in the ACC group and 2.392 kg in the control group. In the Study 2, the ACC supplementation lead to a decreased FCR of 2% in the total period (day 0-42,) with the highest effect in the grower phase (-4%). The final weight (42 days) was increased by 78g in the ACC 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 ACC group compared to control with the highest effect in the finisher phase (-8%). Growth rate followed the same trend with an increase in average daily gain (ADG) of 4%, resulting in a 134g higher final weight (42 days). The highest effect was observed in the finisher phase with a 12% increase in ADG. In conclusion, these studies highlight the capacity of the ACC to improve feed efficiency and growth of broilers with or without 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, Digestibility, Feed efficiency
Metabolism and Nutrition: Feed Additives

158 Evaluation of two types of marine rest raw materials in broiler diets: Effects on live performance and meat lipid quality. Ahmad Fraz*1, 2, Ibrahim A. Khan1, Bing Brackeen1, Gita Cherian2, 1Pacific Seafood, Pacific Bio Products, Warrenton, Oregon, United States, 2Animal and Rangeland Sciences, Oregon State University, Corvallis, Oregon, United States.

The current study investigated the impact of feeding two different marine rest raw materials on live performance, carcass yield, muscle fatty acid (FA) composition, lipid peroxidation products, and blood constituents in broiler chickens. Ninety (n=90), day-old Cobb chicks were fed a corn-soybean meal-based diet with no marine product (Control), 5% deboned white fish meal (Diet 1), or 5% surimi byproduct meal (Diet 2) for 42 days (30 birds per treatment kept in six replicate pens with 5 chicks per pen). For bird performance (feed consumption, weight gain), each replicate pen was considered as the experimental unit. For carcass yield, blood constituents, and lipid analysis, two chickens collected from each replicate is considered as the experimental unit. Bodyweight gain and feed:gain were determined at the starter (1-10), grower (11-21), and finisher (22-42) phases. For all response variables, the effect of marine byproduct vs Control, and Diet 1 vs. Diet 2 were compared separately using analysis of variance. P-values were considered significant at ≤ 0.05. No difference was observed in the body weight gain and feed:gain at day 10 (P>0.05). At day 21, Diet 1 and Diet 2 chicks were higher in body weight than Control (P< 0.05). At day 42, Diet 1 chicks were highest in body weight (3018 g) when compared to Control (2728 g) (P<0.05) and was not different from Diet 2 (2912 g) (P>0.05). Overall, feed:gain was lowest in Diet 2 (1.51) (P<0.05). The relative organ weight (liver, fat pad, heart, and gizzard) and yield of breast and thigh muscles were not different (P>0.05) among the three treatments. No difference was observed in moisture and cholesterol content in breast and thigh muscles (P>0.05). The total fat content of thigh muscle was lower in Diet 1 and Diet 2 (3.4%, 3.3%) compared to Control (4.4%) (P<0.05). No difference was observed in the total fat content of breast muscle (P>0.05). Blood cholesterol was lower in Diet 1 than Control (P<0.05). Feeding Diet 2 led to reduction in blood calcium with a concomitant increase in blood phosphorus compared to Diet 1 and Control (P<0.05).

Total long chain (>20C) n-3 fatty acids were 1.5, 5.5 and 8.2% and 0.5, 2.6 and 3.7% in the breast and thigh muscle of Control, Diet 1 and Diet 2 (P<0.05). Lipid peroxidation products measured as thiobarbituric acid reactive substances (TBARS) were lower in the breast muscle of Diet 1 and Diet 2 compared to Control (P<0.05). However, no effect of diet on thigh muscle TBARS was observed (P>0.05). As consumer demands for n-3 FA-rich poultry products are on the rise, marine-based products may serve as feed supplements that could be used in broiler diets for enriching edible tissues with n-3 FA while increasing bird live performance.

Key Words: broiler, marine rest raw materials, n-3 fatty acid, lipid peroxidation products

159 An algae-derived feed ingredient positively impacted broiler growth performance in the grower phase but did not alter intestinal integrity. Meaghan M. Meyer*1, 2, Elizabeth A. Bobeck, Animal Science, Iowa State University, Ames, Iowa, United States.

Bioactive phytogenic feed ingredients are increasingly incorporated into poultry rations to improve health and performance. Prior work has reported that algae-based ingredients specifically improve broiler gut health parameters, but in vivo studies are lacking. The objective of this work was to measure the impact of an algae-derived feed ingredient (ZIVO Bioscience, Keego Harbor, MI) on the performance and gut permeability of a commercial broiler line fed through the starter and grower phase. Two replicate studies of 200 Ross 708 broilers each (n=400 birds total) were carried out using chick brooder batteries (n=5 birds/cage) for 28 days. Birds were randomly allocated to either a standard diet containing no feed additive (CON), or the standard diet supplemented with 0.175% algae product (ALGAE), and were fed ad libitum d0-27. On d27, all birds were individually weighed, and half of the pens of each group were subjected to a 12-h feed restriction (FR) gut integrity challenge. On d28, fluorescein isothiocyanate dextran (FITC-d; 3,000-5,000 molecular weight) was administered by oral gavage as an indicator of gut leakage. Blood was drawn 1h post-FITC-d gavage and serum collected for analysis. Data were analyzed using PROC MIXED in SAS version 9.4 (Cary, NC). Performance data were analyzed with the fixed effects of diet, and FITC-d fluorescence data were analyzed with the fixed effects of diet, FR, and the diet by FR interaction. No performance differences were detected in the starter period (d0-14), but the ALGAE treatment group showed an increased average weight gain in the grower period (d14-27) by 0.05 kg (P=0.005) and overall (d0-27, P=0.034) compared to the CON. This positive effect of algae inclusion was reflected in improved average daily gain in both the grower period (3.7 g/d, P=0.01) and overall (1.7 g/d, P=0.048). No significant differences were detected in body weight, feed intake, or FCR in either performance period. Under our research conditions, the beneficial mechanism of algae on growth may not be directly related to gut barrier integrity, as the ingredient did not display a protective effect on intestinal barrier function following the 12-h feed restriction challenge. While the feed restriction itself was successful in stressing the gut and triggering translocation of FITC-d beyond the non-fasted control (FR P<0.0001), there were no differences detected based on diet (P=0.874) or diet by FR interaction (P=0.979). An explanation for this outcome is
likely due to the unchallenged health status of the birds and the relatively clean brooder cage environment. Future research in this area is warranted to study this phytochemical ingredient in an immune-challenged flock or in a commercial setting.

**Key Words:** broiler, additive, gut health, performance, algae

160 Chitosan oligosaccharides as potential antibiotic replacements in broiler diets. Emanuele C. Goes*#", Yao Huang, Lingyun Chen, Doug Korver, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada.

Necrotic enteritis (NE) is an enteric disease caused by *Clostridium perfringens*, which can result in severe economic losses in broiler chicken production. The move to reduce antibiotic growth promoters (AGP) in animal diets has hindered the control of NE in broilers and effective replacements must be found. Dietary shellfish chitosan oligosaccharides (COS) were screened as potential AGP replacements in broilers from 0 to 38 d. A total of 1,040 birds were randomly distributed across 26 treatments with 5 replicates of 8 birds each. Treatments were COS at 8 different molecular weights (220, 180, 110, 95, 30, 25, 17 or 14 KDa) at three dietary levels (0.2, 2 or 5 g/kg), plus 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 an oral 15X 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, at the end of the starter, grower and finisher phases, respectively. Data were analyzed by ANOVA with means compared by Tukey’s test at 5% probability. The results presented focus on treatments that had indications of recovery or partial recovery of performance relative to the NC group. After the challenge, the PC treatment had BWG of 44.98 ± 2.25 g/bird per day and FI of 73.30 ± 4.02 g/bird per day vs. BWG of 37.2 ± 2.59 g/bird per day and FI of 62.30 ± 4.64 g/bird per day from NC group. The COS 95, 25 and 17 KDa treatments allowed a partial recovery of growth performance following the challenge. The 95 KDa COS at 0.2 or 5 g/kg partially restored the BWG (10.2 and 8.4% greater than NC, respectively, P = 0.02), and all inclusion levels of this product restored FI (14.9, 3.9 and 15.3%, respectively, P = 0.01) after the challenge. The 25 KDa COS at 5 g/kg increased the BWG by 2.7% and FI by 14.17% (P < 0.01), whereas 2 g/kg only increased FI by 1.9% (P < 0.01) relative to NC after the challenge. The 17 KDa COS at 0.2, 2 and 5 g/kg increased BWG by 1.9, 8.6 and 7.3%, respectively, after the challenge relative to NC (P = 0.04), but had no effect on FI. The 95, 25 and 17 KDa COS showed no effects on FCR regardless of the dose. The recovery of performance may be related to the potential of COS to prevent pathogen colonization, enhance immune response or act as a prebiotic. COS may be part of a strategy to replace AGP, however, further study is required to understand the mechanism of action.

**Key Words:** Broiler, chitosan oligosaccharide, antibiotic replacement, growth performance, necrotic enteritis

161 Evaluating the effects of a novel phytogenic feed additive on performance of broilers fed a low or standard protein diet. Chasity M. Pender*, Shelby Ramirez², G. R. Murugesan¹, Eric B. Sobotik³, Gregory S. Archer², ‘Biomin America Inc., Overland Park, Kansas, United States, ²Department of Poultry Science, Texas A&M University, College Station, Texas, United States.

Demand for dietary crude protein (CP) is high in fast-growing modern broilers as protein is required for rapid growth and immune system development and response. Phytochemical feed additives (PFA) have shown to increase dietary protein utilization with subsequent benefits on performance. The objective of this study was to evaluate the effects of a novel PFA formulation as well as other commercially available PFAs on performance of broilers provided a standard (SP) or low protein (LP) diet. A total of 1,260 day-old Cobb 500 chicks were randomly allocated to one of seven treatment groups; SP: a standard control provided a basal diet containing adequate levels of CP, LP: a reduced control provided a basal diet containing a 1.5% reduction in CP compared to the SP diet, YUC1: LP + PFA composed of *Yucca Schidigera* and *Quillaja saponaria*, YUC2: LP + PFA derived from *Yucca Schidigera*, BLEND: LP + PFA composed of a blend of essential oils, herbs, spices, and saponins, EO: LP + PFA containing a mixture of essential oils, or PEPY: LP + Digestarom® PEP-Y. Each group consisted of 10 replicate pens (18 birds/pen). The trial was conducted over a 35-day period and performance variables were calculated weekly as cumulative measures. Data were analyzed using the GLIMMIX procedure of SAS with significance reported at P ≤ 0.05. Reduction in CP significantly reduced BW and BWG for the DOH-d21 and DOH-d28 periods, while FCR was increased for the DOH-d21 period compared with birds fed SP diet. For DOH-d21, BW, BWG, and FCR of PEPY was intermediate between the SP and LP controls. During the same period, BW and FCR of YUC1, YUC2, BLEND, and EO was impaired compared with SP and similar to LP. A similar trend was seen for BWG, except BLEND and PEPY were intermediate to the two controls, while the remaining groups were reduced compared to SP and similar to LP. For DOH-d28, BW and BWG of PEPY was intermediate between SP and LP, while the other PFA supplemented groups were similar to LP and reduced compared to SP. Feed conversion was numerically increased in LP, YUC1, and PEPY compared to SP, whereas YUC2, BLEND, and EO had significantly increased FCR compared to all other groups. For the overall DOH-d35 period, FCR was significantly increased in YUC2, BLEND, and EO compared to both controls and PEPY, while YUC1 was intermediate. Overall, supplementation of Digestarom® PEP-Y improved performance and offset the negative effects of lower CP.

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inclusion in the diet suggesting augmented protein utilization towards growth.

Key Words: Crude Protein, Broilers, Phytogenics, Performance

162 Sweet potato (*Ipomoea batata*) as natural additive: effect on productive performance of Creole chickens of Mexico in initiation phase. Cesar A. Pérez-Torres,1,2 Diana A. Gutiérrez Arenas1, Arturo Pro-Martínez2, Eliseo Sosa-Montes1, Leonardo Osio-Orhiuela1, Vanessa R. Lavastida1, Luis Morales-Higuera1, Fernando González-Cerón1, ‘Animal Science Department, Chapingo Autonomous University, Texcoco, State of Mexico, Mexico, 2College of Postgraduates, Texcoco, State of Mexico, Mexico, 3Life of Sciences Division, University of Guanajuato, Irapuato, Guanajuato, Mexico.

Beta-carotene is an antioxidant compound. Sweet potato is rich in beta-carotene, so its inclusion in feed could improve the productive performance of meat birds. The aim of this study was to assess the effect of sweet potato as natural additive on productive performance of Creole chickens of Mexico during the initiation phase (0 to 21 days of age). A total of 105 straight run 0-d-old Creole chickens were randomly allocated to three treatments (five replicates per treatment and seven birds per replicate): 0-SPF, 0 mg of sweet potato flour kg⁻¹ of feed; 500-SPF, 500 mg of sweet potato flour kg⁻¹ of feed, and 1000-SPF, 1000 mg of sweet potato flour kg⁻¹ of feed. Birds were raised in battery brooders and fed a diet containing 20.5% CP and 2,900 kcal ME kg⁻¹ of feed. Water and feed were provided *ad libitum* during the 21 d of the experiment. Sweet potato was dried by thermic treatment at 40°C for 24 h, and then it was milled to obtain the flour. Feed consumption and body weight were recorded weekly in order to estimate daily feed intake (DFI), daily weight gain (DWG), feed conversion ratio (FCR), cumulated weight gain (CWG) and final weight (FW). Data were analyzed using the MIXED procedure of SAS. Birds fed 0-SPF and 1000-SPF diets had better (P < 0.05) DWG from 7 to 14 d of age (10.8±0.3 and 10.7±0.3 g, respectively) with respect to chickens fed 500-SPF diet (9.8±0.3 g). In contrast, during the period from 14 to 21 d of age, the 500-SPF and 1000-SPF birds showed greater (P < 0.05) DWG (12.1±0.5 and 12.7±0.5 g, respectively) compared to chickens in 0-SPF group (10.3±0.5 g). FCR was not different (P ≥ 0.05) among treatments from 0 to 7 and 14 to 21 d of age. However, from 7 to 14 d of age, birds in 1000-SPF treatment had a lower (P < 0.05) FCR value (1.63±0.044) compared to 0-SPF and 500-SPF chickens (1.698±0.044 and 1.784±0.044, respectively). No differences (P > 0.05) were observed among 0-SPF, 500-SPF, and 1000-SPF treatments in terms of CWG (193.5±5.8, 198.2±5.8, and 211.6±5.8 g, respectively) and FW (242.2±5.5, 243.9±5.5, and 258.7±5.5 g, respectively). In conclusion, these results suggest that the inclusion of sweet potato flour as natural additive in feed of Creole chickens of Mexico, could improve DWG from 14 to 21 d of age and FCR from 7 to 14 d of age.

Key Words: Creole chickens, Nutraceutical feeds, *Ipomoea batata*, Antioxidant, Mexico

163 Not Presented.

164 Effects of black soldier fly larvae oil on growth performance, gut physiology and fatty acid composition of broiler chickens. Yoo Bhin Kim1,2, Da-Hye Kim1, Sang Hyeok Lee1, Tae-hoon kim1, Hong gu ee1, Kyung-Woo Lee2, 1Konkuk university, Seoul, Korea (the Republic of), 2Foody Worm, Cheongju-si, Korea (the Republic of).

This study was carried out to investigate the effect of the black soldier fly larvae (BSFL) oil on growth performance, carcass traits, short-chain fatty acids, fatty acid composition in abdominal fat, and serum parameters in broilers. It is expected that dietary black soldier fly larvae oil would affect growth performance, gut physiology, serum parameter and fatty acid composition in broiler chickens. A total 450 one-day-old male broiler chicks (Ross 308) were randomly allocated to three treatments with 10 replicates. A corn and soybean meal-based diet was used to formulate three experimental diets containing corn oil, coconut oil and BSFL oil at the level of 50 g per kg of diet. Data were analyzed by ANOVA using the PROC GLM followed by Duncan’s multiple range test in SAS. None of dietary fat sources affected body weight gain and feed intake in broiler chickens during days 1 to 30. Feed conversion ratio was decreased (P < 0.05) in the coconut oil and BSFL oil groups compared with the corn oil group. Ileal weight-to-length ratio significantly elevated (P < 0.05) in BSFL oil group compared with corn and coconut oil groups in 30-day-old broilers. The pH of the breast meat was lowest (P < 0.05) in the coconut oil group compared with that in the corn oil and BSFL oil groups. Branched-chain fatty acids in ileal contents were higher (P < 0.05) in chickens fed the BSFL oil group compared with those fed either corn or coconut oil group at 15 days. On the other hand, ileal propionate contents were higher (P < 0.05) in the coconut oil-fed group compared with the corn and BSFL oil groups at 30 days. BSFL and coconut groups had higher proportions (P < 0.05) of saturated fatty acid being dominant in lauric and myristic acids in the abdominal fat compared with the corn group. On the other hand, unsaturated fatty acids including oleic acid, linoleic acid, and arachidonic acid were higher (P < 0.05) in the corn oil group than in coconut and BSFL oil groups. BSFL oil group lowered (P < 0.05) concentrations of total cholesterol in serum samples compared with corn and coconut oil groups at 30 days. BSFL oil group increased (P < 0.05) total antioxidant capacity in serum samples compared with corn oil and coconut oil groups at 30 days. In conclusion, the present study suggests that dietary BSFL oil improve feed conversion ratio and increase the incorporation of medium chain fatty acids into abdominal fat in broiler chickens.

Key Words: Black soldier fly larvae, broiler chickens, fatty acid composition, growth performance, gut physiology
An experiment was conducted to evaluate the effects of Optiphos and a new intrinsically heat tolerant product, Optiphos Plus when supplemented to broiler starter diets, on live performance and bone mineralization. Male newly hatched Cobb-500 broiler chicks (n=576) were randomly allocated to 8 replicate stainless steel battery rearing pens and fed 1 of 12 different diets for a 21 day period. There were two control diets, a negative control (NC) containing 0.17% non-phytate phosphorus and 0.88% calcium and a positive control (PC) containing 0.37% non-phytate phosphorus and 1.08% calcium. Analysis of total calcium and phosphorus were in agreement with expected levels. Treatments were created by supplementing the NC basal diet with one of the phytase sources at 5 different levels to illicit large responses to the phytase sources. The remaining 10 treatments were created by supplementing the NC basal diet with one of the phytase sources at 5 different levels to include 125, 250, 500, 1000, or 1500 FTU/kg. All diets were analyzed for phytase activity and demonstrated that correct levels of each source were included. Experimental treatments were arranged as a 2 X 5 full factorial using a randomized block design and analyzed using the Least Squares Procedure of JMP 13.1 and regression analysis. The NC and PC groups serves as our control references with a breaking strength of (26.1 ± 0.7g/kg) and percent bone ash of (42.1 ± 0.5) when compared to the PC which were: starter (d 1 to 16; S), grower (d 17 to 29; G), and finisher (d 30 to 43; F). On d of hatch, male Ross 708 X Yield Plus broiler chicks (n = 700) were weighed and randomly assigned to 28 floor pens with 25 birds per pen for a total of 14 replicate pens per treatment. Feed and water were provided ad libitum and environmental conditions were monitored throughout the experiment. Feed intake (FI) and individual BW were recorded to evaluate the FI, BW gain, and calculate feed conversion ratio (feed:gain; FCR) performance at the end of each feed phase. The 2 median BW birds per pen were selected to obtain blood samples for CC analysis at 43 d of age. Data were analyzed using a generalized linear mixed model with the GLIMMIX procedure of SAS and least square means were separated at P ≤ 0.05. There were no differences in BW during any feed phase (P ≥ 0.2903). No differences in FI or FCR were observed among treatments during S and F phases (P ≥ 0.2323). However, during the G phase, broilers fed the CON diet had lower FCR compared to CCD (P ≥ 0.0294). Cumulatively (d 1 to 42), there were no differences in FI, BW gain, or FCR among treatments (P ≥ 0.1979).

According to the blood carotenoid analysis, the CCD-fed birds had similar blood CC compared with CON-fed birds.
(P ≥ 0.8968). In conclusion, the addition of CD to the diet of broilers did not result in improved broiler blood CC or growth performance, except for the G phase where CON-fed broilers had lower FCR.

**Key Words:** essential oils, carotenoid concentration, broiler growth performance, body weight, poultry production

168 Not Presented.

169 The effect of Viligen® on performance and intestinal morphology of the broiler birds exposed to heat stress during d21- d42 of the finishing period. Manoj B. Kudupoje*, 1, 2, Daniel Graugnard2, Alexandros Yiannikouris2, Malathi V1, 1Poultry Science, KVAFSU, Bangalore, Karnataka, India, 2Center for animal Nutrigenomics & Applied Animal Nutrition, Alltech Inc., Nicholasville, Kentucky, United States.

Research works have documented the negative effect of heat stress on intestinal barrier integrity and functions by damaging intestinal epithelium. Studies in monogastric animals have shown that organic acids, such as butyric acid, promote gut health and integrity thereby improving nutrient absorption and productive performance. However, organic acids blended with other feed additives may yield a different response. In this study, butyrate blended with prebiotics and organic minerals (Viligen® Alltech, Inc.) was evaluated in broilers under heat stress challenge. A total of 800 non-vaccinated one-day-old male Cobb broiler chicks were distributed according to a CRD into four treatments with 8 replicates (25 chicks/replicate). T1: control – No heat treatment, T2: control – No heat treatment + 0.10 % Viligen®, T3: Heat treatment (d21-42; 32 °C; 64 % RH) and T4: Heat treatment (d21-42; 32 °C; 64 % RH) + 0.10 % Viligen®. Birds were raised on floor pens and provided NRC recommended, iso-nutritive experimental diets ad libitum without antibiotic or coccidiostat. On d42, 2 birds from each pen were evaluated for small intestine villus height (VH), crypt depth (CD), VH: CD ratio, and goblet cell count. Data were subjected to ANOVA (GLM procedure of SPSS) with means separated using Fisher’s LSD test. Viligen® supplemented birds had higher body weight (P<0.05) on d28 and d42 irrespective of heat stress. Moreover, lower FCR (P <0.01) on d 35 and d42 was observed in broilers provided Viligen® supplementation with or without heat stress. During heat stress jejunum VH and VH:CD ratio were higher (P<0.05) in birds supplemented with Viligen®, while in non-heat-stressed birds ileum VH and VH: CD ratio was greater (P<0.05) in birds supplemented with Viligen®. There was a higher goblet cell count in the duodenum (P<0.05) of the non-heat stressed Viligen® supplemented group compared to both heat-stressed groups. Architectural modulation of the small intestines, i.e., the increased villus length and the corresponding surface area, is related to better absorption of nutrients. Based on these results, Viligen® supplemented broilers had better gut health and performance under heat stress.

**Key Words:** Butyrate, heat stress, broilers, gut health, Viligen

170 Dietary supplementation of alpha-lipoic acid mitigates the negative effects of heat stress in poultry. Sanjeev Wasti*, Nirvay Sah, Chin N. Lee, Rajesh Jha, Birendra Mishra, Department of Human Nutrition Food and Animal Sciences, University of Hawaii at Manoa, Honolulu, Hawaii, United States.

Heat stress accounts for substantial economic loss in the poultry industry by altering health and performance of chickens. Alpha-lipoic acid (ALA) is a water and fat-soluble antioxidant which can readily get absorbed from the intestine resulting in maximum bioavailability. Moreover, ALA acts as a coenzyme in glucose metabolism and helps generate other antioxidants. Considering these benefits, we hypothesized that dietary supplementation of ALA would help mitigate heat stress in poultry. Day-old broiler chicks (n=72) were randomly assigned into three treatment groups (n=24/group): no heat stress (NHS), heat stress (HS), and heat stress with alpha-lipoic acid (HS+ALA) and reared under standard husbandry practices for 3 weeks. Afterwards, birds in the HS and HS+ALA groups were exposed to heat stress (33°C for 8 hours during daylight) for 3 weeks, while the NHS group was reared under normal condition (22°C-24°C). The HS+ALA group received feed fortified with ALA (500 mg/kg) during the treatment period, while other birds were provided with the standard broiler diet. Weekly body weight and feed intake were recorded. The cecum digesta for volatile fatty acids (VFAs) analysis and 16S rRNA sequencing for the gut microbiota analysis; and the ileum tissue samples for histological and gene expression analyses were collected on day 42. The difference between treatment groups were analyzed using one-way ANOVA followed by the Tukey-Kramer test with a significance of P<0.05. Exposure to heat stress decreased (P<0.05) average daily gain and final body weight in HS group compared to NHS group, whereas supplementation of ALA significantly (P<0.05) improved these factors in heat-stressed birds. Furthermore, birds in the HS+ALA group had increased (P<0.05) expression of HSP90, PRDX1, GPX3, SOD2, OCLN, and MUC2 genes and higher (P<0.05) concentration of major VFAs (acetate, propionate, and butyrate) compared to HS group. The ALA supplementation also improved the villus height and villus height to crypt depth ratio in the HS+ALA birds. The microbial diversity analysis revealed significant abundance (P<0.05) of beneficial bacteria Lactobacillus and Peptostreptococcaceae in the cecum. Taken together, these results indicate that dietary supplementation of ALA is effective in partially mitigating the negative effects of heat stress by improving villus structures, increasing the concentration of major VFAs, enriching the beneficial microbiota, and enhancing the expression of antioxidants, tight junction, and immune-related genes in the intestine.
Thus, ALA supplementation can be considered as a potential strategy to ameliorate the effects of heat stress in poultry.

**Key Words:** alpha-lipoic acid, gene expression, gut microbiota, heat stress, mitigation

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**171 Effect of two limestone sources (fine, coarse) and a novel consensus bacterial 6-phytase variant on ileal P and Ca absorption and tibia ash content of young broilers.** Cees Kwakernaak¹, Yueming Derjant-Li²*, Abiodun Bello², ¹Poultry Nutrition, Schothorst Feed Research, Lelystad, Netherlands; ²Nutrition & Biosciences, DuPont, Oegstgeest, South Holland, Netherlands.

There is growing interest for the effects of limestone particle size (LPS) and the respective Ca solubility (Ca-sol) on phytase efficacy and nutrient digestibility. It is hypothesised that a coarser LPS can result in a higher phosphorus (P) and calcium (Ca) absorption (abs). A digestibility study was carried out to determine the efficacy of a novel consensus phytase variant and the influence of two limestones with different Ca-sol. Male Ross 308 broilers (n=720) were used in a 2x5 factorial arrangement (6 balance cages of 12 birds per treatment) with two LPS and 5 phytase dose levels in corn-based diets. A negative control diet (NC) was formulated without an inorganic P source (2950 kcal/kg AME, 200g/kg CP, 2.8 g/kg phytate-P, 4.4 g/kg P and 5.1 g/kg Ca). The two LPS (both with 38.5% Ca) were 0.3-0.6 mm (coarse) and <0.1 mm (fine) with Ca-sol (after 5 min at pH=3) at 42 and 97%, respectively. The phytase was added as pellets from 11 to 21 days of age. On day 21, ileal digesta samples of all birds and tibia (4 birds/cage) were collected and analysed for Ti, Ca, P and bone ash content in fat free dry matter. Absorbed P, Ca and tibia ash were analysed via 2-way ANOVA (P<0.05 considered as significant). Significant interaction between LPS and phytase dose was present on all 3 parameters. At all dose levels, abs P was higher with coarse vs fine LPS. From the 1,000 to 2,000 FTU/kg dose, abs P was further increased (P<0.05; 0.31 for 1,000 and 2,000 FTU/kg to NC diets. Each phytase dose improved abs P in an exponential manner regardless of LPS, while the abs P is greater with coarse LPS, the improvement due to phytase is greater with fine LPS. Likely, abs Ca was the limiting factor for the lower tibia ash content with the lower Ca-sol LPS.

**Key Words:** Broilers, Phytase, Ca absorption, P absorption, Limestone Ca solubility

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**172 Use of quantile regression model to evaluate the effects of hydroalcoholic extract of leaves and stems of *Capparis spinosa* on final weight of Ross broilers.** Pouyan Malekinezhad³*,¹, Laura Ellestad¹, Moslem Bashtani¹, Seyed Homayoun Farhangifar¹, Hadi Sarir², ¹Poultry Science, University of Georgia, Athens, Georgia, United States; ²Animal Sciences, University of Birjand, Birjand, Iran (the Islamic Republic of).

Today, plant-based additives have become increasingly utilized as antibiotic alternatives in poultry diets. *Capparis spinosa* (CS) is a plant that can serve as a rich source of antioxidants in the form of flavonoids and tocopherols. The purpose of this research was to evaluate the effects of hydroalcoholic extract from leaf and stem of CS in Ross broiler chicken diets using a quantile regression model. The hydroalcoholic extract was prepared from shade dried CS leaves and stems in a 3:1 ratio, using a 1:1 water: alcohol solution. A total of 700 male day-old chicks were utilized in a completely randomized design with 10 treatments, with 7 replicates and 10 observations in each replicate. The treatments containing 0, 100, 200, 300, 400, 500, 600, 700, 800, and 900 mg of CS extract per kilogram of the diet were used from hatch through 42 days of age, after which birds were weighed. Through fitting a quantile regression model, the regression coefficient of final weights based on the amount of the extract was estimated using SAS software. The results of the analysis of variance and mean comparisons revealed that birds fed 700 and 900 mg/kg of the extract were significantly heavier than the control birds (P<0.05). Moreover, the quantile analysis showed a greater changes in final body weight for birds given the extract in its lower quartiles (P<0.05), while in the higher quartiles a reduced change was observed (P>0.05). This may indicate that the extract does not equally affect different final body weights of the broilers even within the same treatment. Regarding the greater changes of the final weight of the lighter birds, it could be concluded that the extract used in the present research had a greater influence on chicks that were intrinsically smaller during the period of rearing and helped them come closer to meeting their full genetic potential.

**Key Words:** alcoholic extract, body weight, Capparis spinosa, flavonoids, quantile regression

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**173 Improved performance of turkey toms administered two phytopgenic feed additive blends throughout grow-out.** Emily Kimminau*,¹, Theodore Karnezos¹, Nicholas Evans², Curtis Novak², ¹Land O’ Lakes, Williamsburg, Virginia, United States; ²Purina, St. Louis, Missouri, United States.
Missouri, United States.

With fewer anticoccidials cleared for turkeys, producers are faced with difficulty in selecting drug rotation programs. Because of the timelines and costs to get new anticoccidial chemicals approved, it is unlikely for more drugs on the horizon. Therefore, utilizing alternatives to improve performance could extend the use of current anticoccidials. Therefore, we wanted to evaluate Tom performance when fed two phylogenetic blends in addition to a control diet. 2,624 Hybrid toms were placed into 32 pens of 82 poults (10 replications for Treatment 1, 11 replications for Treatment 2 & 3) with built up litter in a commercial research facility in Indiana. Birds were given feed and water ad libitum for the duration of the trial. There were three treatments: Control- Lasalocid (Avatec) 91 g/ton D0-36, Lasalocid 81 g/ton D37-63 and no additives D64-133, 2- Control diet plus Phytogenic blend A 1 lb/ton D0-63, Phytogenic blend B 1 lb/ton D64-133, 3- Control diet plus Phytogenic blend C 1 lb/ton D0-63, Phytogenic Blend D 1lb/ton D64-133. Body weight (BW) and feed conversion (FCR) were measured on D36, 63 and 133. Performance was analyzed using ANOVA. Significant means were separated with least significant differences post-hoc. No significant differences between treatments were seen in mortality or FCR throughout the trial. Treatment 2 and 3 were heavier than Control birds on D36 (P<0.0001). Treatment 2 and 3 were heavier than Control birds on D63 (P<0.002). No significant differences were observed on D133. In conclusion, adding phylogenetic blends improved Tom BW on D36 and D63 with addition of ionophore alone.

Key Words: turkey, phyogenic, feed additive, performance

174 The effect of a synbiotic applied as a coarse spray at the hatchery versus early drinking water application on broiler chick performance and intestinal morphology. Elli Dearmont*,¹, Sheila Purdum¹, Kathryn Hanford²,¹Animal Science, University of Nebraska, Lincoln, Nebraska, United States, ²University of Nebraska, Lincoln, Nebraska, United States.

Antibiotic resistance is a threat to human and animal medicine, causing major restrictions on antibiotic use in broiler production. Synbiotics are being investigated as alternatives to antibiotics by supporting overall bird growth and gut health. A study was conducted to examine the effects of a synbiotic on production parameters and early intestinal morphology of broilers using a 2x2 factorial arrangement. The four treatments utilized were: 1. no synbiotic applied, 2. synbiotic applied only as a coarse spray on the day of hatch, 3. synbiotic applied only in the drinking water for the first 3 days, and 4. synbiotic applied as both a coarse spray on day of hatch and in the drinking water for first 3 days. The product was prepared according to the manufacturer’s specifications where each chick received 1x10⁸ CFU/day. The synbiotic contained fructooligosaccharides and the bacterial species Lactobacillus reuteri, Bifidobacterium animalis, Pediococcus acidilactici, and Enterococcus faecium. Sixteen flocks of Ross 708 broilers were raised in commercial broiler houses for 7 weeks. Each treatment was randomly assigned to four flocks/treatment. First week mortality (FWM) and body weights (BW) at 7 and 49 days of age were measured. FWM for treatment 1 was 1.18%, for treatment 2 was 1.34%, for treatment 3 was 1.19%, and for treatment 4 was 1.22%. Samples of jejunum tissue were taken on 3 and 10 days of age. Villi height, crypt depth, and villi: crypt ratio were measured. A novel scoring system was used to quantify the extent of epithelial sloughing and damage observed on villi tips. Scores from 1–4, where 1 is least damaged and 4 is most damaged, were assigned. Statistical analyses were performed using the GLIMMIX procedure of SAS. The model used included the fixed effects of spray and water and their interaction. Fisher's protected LSD was used for all comparisons among the least squares means using an alpha <0.05. No significant effect of the interaction between spray and water application was detected for any parameter. There were no significant treatment effects (p>0.05) on FWM, BW, villi height, crypt depth, or villi: crypt ratio. The treatment applied as a coarse spray did not significantly affect jejunum epithelial scores. However, the synbiotic treatment administered in the drinking water significantly increased epithelial damage observed on villi tips using the novel scoring system (p<0.05) suggesting, it potentially resulted in less nutrient digestion and absorption in the jejunum. In conclusion, although more extensive villi damage was observed when synbiotic was administered in drinking water, it did not affect broiler growth and productivity.

Key Words: broiler, synbiotic, performance, intestinal morphology, villi score

175 Evaluation of a novel proprietary feed additive to strengthen or substitute avilamycin growth promoter in broiler standard program. Bertrand MEDINA¹, Dana KUMPRECHTOVA¹, Ashley Wagner*¹, Vlastislav Machander, Ivan D. Girard¹, Probiotech International Inc., St-Hyacinthe, Quebec, Canada, ¹International Poultry Testing (MTD), Ustrasice, Czechia, ¹Institute of Animal Science, Prague, Czechia.

Depending on their importance for human medicine, the preventive use of antibiotics was progressively prohibited in standard feeding programs for broilers in North American countries. Unclassified, avilamycin (Avil.) will soon remain the only authorized prophylaxis molecule to cope with Necrotic Enteritis syndrome (NES). The objective of this study was to test the efficacy of a novel, proprietary feed additive (Alterna®Poultry (AP), a blend of phylogenics, organic acids and functional oils) formulated by Probiotech International Inc. to support broiler performances reared under standard feeding program. One-day old ROSS 308 chicks (n=600) were randomly assigned to 3 treatments: a medicated group (T1) with Avil. at 15 ppm in pre-starter and starter (1-21d); a mixed-program group (T2) with AP at 400
ppm in pre-starter (1-7d), Avil. at 15 ppm in starter and grower (8-29d) and AP at 300 ppm in finisher and a natural group (T3) with AP at 400 ppm from pre-starter to grower (1-29d) and 300 ppm in finisher. Each treatment was fed to 5 replicates of 40 mixed-sex broilers. Diets were in pellet form. A chemical coccidostat (diclazuril) was used in pre-starter and starter phases and rye (5%) was included as a low intestinal challenge. Individual live weights (LW) were recorded after 7, 21, 29 and 42 days. Feed intake (FI) was measured for each feeding phase, dead birds were counted daily and reported as cumulative percentage. At 25 days, bacterial enteritis (BE) was scored (1 M + 1 F / pen) as described by De Gussem (2010). At 42 days, total foot pad lesions (FPD) was ranked according to a 0 - 4 scoring chart. Treatment means were compared with a single-factorial model ANOVA. Means were further separated using a Duncan’s LSD test and P<0.05 as significant threshold. No model ANOVA. Means were further separated using a lowered by the presence of AP in both T2 and T3 groups culled (1%) in T3 group. At day 25, the BE score was significantly affected by treatment (P>0.05; T1: 2735; T2: 0.73a; T2: 0.53b and T3: 0.68ab). Over the 42d trial, bird performances (LW, FI and FCR) were not significantly affected (P<0.05; T1: 2,21b; T2: 1.49a and T3: 1.57a ). The results indicate that the alternating use of AP may strengthen a preventive use of avilamycin to cope with NES. Moreover, under these experimental conditions, this novel proprietary feed additive appears as a natural alternative as efficient as avilamycin (15 ppm) for maintaining broiler performances and also securing one of the bird welfare parameters (FPD).

**Key Words:** phytogenic, functional oils, Avilamycin, Foot pad lesions, broiler

176 The effects of yeast fermentate prebiotic feed additive on the lactic acid producing bacteria in an in vitro microaerophilic cecal culture model. Lindsey A. Wythe, Kristina M. Feye, Dana K. Dittoe, Steven Ricke

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Investigating new tools to improve food animal health and production is essential to safeguard the food supply. ProBiotein, a yeast-fermentate prebiotic compound derived from Brewer’s yeast, has been used successfully in cattle management programs to improve gut health and feed efficiency. Developing this tool for poultry first requires understanding the dose-response of the yeast fermentate, which can be linked to changes in the microbiota. Because anecdotal evidence suggests ProBiotein may be useful as a poultry feed supplement, the following study was conducted to determine the effects of including ProBiotein in chicken feed on the cecal microbiota. A total of 10 chicken ceca from commercial broilers were collected and subjected to a microaerophilic in vitro ceca culture model. The ceca were subjected to the following treatments: 0, 0.5, 1, 1.5, 2, or 3% w/v ProBiotein with time collections at 0 and 24 h post-treatment with 0 h post-culture initiation (N=60, k=6). Immediately following the addition of the ceca culture to the individual treatments, bottles were mixed, pressurized to the standard gut pressure (5 to 6 PSI) using microaerophilic gasses, and incubated at 37 °C for 24 h at 150 rpm. Ceca cultures were evaluated for LAB and APC load changes over time. The CFU counts were Log10-transformed, with data analyzed using two-way ANOVA in JMP 15.0 with means separated using Tukey’s Protected HSD (P < 0.05). The results of the current study demonstrated a significant interaction of treatment and time, as well as the main effects for LAB populations (P < 0.05). There were no differences between the treatments for APC load, and there was no difference between the treatment groups at 0 h with a mean of 5.8 Log10 CFU/g (P > 0.05). At 24 h post-treatment, 3% ProBiotein had the least amount of LAB bacteria at 9.16 Log10 CFU/g compared to the other groups (P < 0.05). All groups demonstrated at least a 4 log10 CFU/g increase in LAB load between 0 and 24 h (P < 0.05). The 2% ProBiotein treatment group had significantly lower LAB loads than the 0%, 0.5%, and was not different from the 1% group. The 0.5% and 1.5% was no different than the NTC (P > 0.05). Based on data presented, 3% ProBiotein demonstrates the greatest decrease in LAB load as compared to the other treatment groups, while 0.5% through 1.5% have the greatest increase in LAB populations. Additional studies evaluating the microbial ecology will need to be conducted to understand the effects these populations have on the microbiome.

**Key Words:** Fermentate, Yeast, LAB, Poultry, Ceca

177 The impact of dietary Beta-Glucans on poultry health and performance. Luke Trimble, Arianna Ferguson, Brooke Migdal, Max Pasquinelli, Nuket Acar, Paul Patterson, Animal Science, Penn State University, University Park, Pennsylvania, United States, Veterinary & Biomedical Sciences, Penn State University, University Park, Pennsylvania, United States.

This study measured immune and performance parameters of broilers fed 2 beta-glucans from mushroom Agaricus blazei, Agaricus Bio CX (AGM), Atlas World USA and yeast Saccharomyces cerevisiae, Original (XPC), Diamond V. 810 broilers (Cobb x Ross) were divided into 3 treatments with 6 replicate pens of 45 birds per pen. Starter, Grower and Finisher diets were formulated to meet nutritional requirements including a Control and 2 treatment diets with either 0.10% AGM or 0.0625% XPC added to the manufacturer’s recommendations. Bursa of Fabricius weight, cellular immunity via PHA-P toe injection and humoral immunity via SRBC injection were measured 3 times from 3 birds/pen. Pen body weight, feed intake and feed conversion were measured during each diet period (n=42, 39 and 36 birds/pen, respectively). Data were analyzed as a one-factor ANOVA using the GLM procedure of SAS software (SAS, 2012) and Tukey’s comparison for mean separation when the F-test was significant (P<0.05). Results for the Starter period indicate the Control and AGM
were significantly greater compared to the XPC (P<0.05) for feed intake, body weight (208 and 201g vs. 194g, respectively) and body weight gain than the XPC, resulting in a higher feed/gain ratio for XPC. For the Grower period the Control birds were significantly greater than the XPC fed pens (P<0.05) for feed intake, body weight (674 vs. 630g) and body weight gain, while the AGM birds were intermediate and not significantly different. For the Finisher period there was no significant treatment differences for feed intake, body weight, body weight gain and feed/gain ratio. Overall, at 42 d there was no impact (P>0.05) of treatment on gain (mean = 2.017g), feed intake (mean = 3.804g), feed/gain ratio (mean = 1.887), bursa weight or bursa percentage of body weight. For cellular immunity, no significant treatment effect on toe thickness was observed at 0, 24, 48 or 60 h following PHA-P injection. But for the saline injection there was a significantly greater 60 h response for the AGM compared to the Control (P<0.05) and the XPC was intermediate. Similarly, there was a significant increase in right toe thickness from 0 to 24 h post PHA-P injection (P<0.05) at 28 d for AGM compared to the Control, while the XPC was intermediate (P>0.05). Finally, there was no significant impact of treatment on mortality during the 42-d study (mean=2.74%). In conclusion, while birds at both 14 and 28 d of age.

XPC treatments elicited a greater response than the Control, while the XPC was intermediate (P>0.05). Finally, there was a cellular immunity trend indicating the AGM and the XPC was intermediate. Similarly, there was a significantly greater 60 h response for the AGM compared to the Control (P<0.05) and the XPC was intermediate. Similarly, there was a significant increase in right toe thickness from 0 to 24 h post PHA-P injection (P<0.05) at 28 d for AGM compared to the Control, while the XPC was intermediate (P>0.05). Finally, there was no significant impact of treatment on mortality during the 42-d study (mean=2.74%). In conclusion, while there were significant beta-glucan effects on feed intake, body weight, gain and feed conversion early on (10 and 21 d), there were none at the end of the study (42 d). However, there was a cellular immunity trend indicating the AGM and XPC treatments elicited a greater response than the Control birds at both 14 and 28 d of age.

Key Words: Performance, Immunity, beta-glucans, Agaricus blazei, Saccharomyces cerevisiae

178 Effects of Bacillus subtilis on intestinal morphological structure of male broilers with or without coccidial challenge. Sabin Poudel1,2*, Wei Zhai, Department of Poultry Science, Mississippi State University, Starkville, Mississippi, United States.

A healthy and intact intestine is essential for proper physiological function of intestine and subsequent optimal growth. A study was conducted to determine the relationship between the small intestinal morphological structure and development of intestine when probiotic (Bacillus subtilis) was fed and cocci challenge was applied. A total of 416 d-old Ross 708 male broilers were randomly divided into 4 treatments, 2 x 2 (Bacillus subtilis x cocci) arranged in 8 replicating blocks. Diets with or without Bacillus subtilis (1.1x10^6 CFU/kg of finished feed) were fed to the birds. One mL distilled water with or without 20x doses of commercial cocci vaccine (Eimeria acervulina, E.maxima MFP, E. tenella) was orally gavaged to each of the birds on d 14. Small intestine (duodenum, jejunum, and ileum) morphological structure was measured from randomly selected birds from each replicate pen on d 27 and only from jejunum on d 36. A 2-way ANOVA and partial correlation analysis were conducted to understand the treatment effects and relationship of the measured variables. Cocci challenge increased crypt depth (CD) of the duodenum (P = 0.0005) and ileum (P = 0.003), but reduced villus height (VH) to crypt depth (CD) ratio (VH: CD) of duodenum (P = 0.0028) and ileum (P = 0.0411) on d 27. Under cocci challenge condition, there are more multiplication of crypt cells to recover the villus lost during Eimeria spp proliferation, which might have resulted in deeper crypts and lowered the VH: CD ratio. Interestingly, cocci challenge increased VH of ileum (P = 0.0129), but did not affect VH of duodenum and jejunum on d 36. There were no effects of diet and cocci challenge on the intestinal morphology of jejunum on d 36 (22 days post-challenge), which indicated that birds may have recovered from the negative effects of cocci challenge with aging. The VH of ileum on d 27 and VH: CD of jejunum on d 36 were negatively correlated with ileum (P = 0.0217) and jejunum (P = 0.0463) length respectively, indicating longer intestine had shorter VH and lower VH: CD ratio. The CD of duodenum and jejunum was positively correlated with the moderate and severe cocci lesion due to Eimeria acervulina on the respective segment on d 27 and d 36 (P = 0.0144 and P = 0.0016). In conclusion, Bacillus Subtilis was not able to reduce the adverse effect on intestinal morphology caused by the cocci challenge. The intestine morphology was adjusted according to the development of intestine, severity of the cocci challenge, and progress of Eimeria spp life cycle in intestine along with time.

Key Words: Intestinal morphology, Bacillus subtilis, Coccidiosis, Intestine development, Broiler

179 Comparison between phytochemical pentadecylphenol and cocci vaccines on growth performance and serum parameters in broilers. Hanyi Shi1,2,*, Yiran Luo1, Qingqing Deng2, Woo Kim1, Ning Liu1, 1Poultry Science Department, University of Georgia, Athens, Georgia, United States, 2Department of Animal Science, Henan University of Science and Technology, Luoyang, Henan, China.

Pentadecylphenol (PDP), also known as cardanol, is a minor constituent of lipids in cashew nut with antioxidant properties. An experiment was conducted to evaluate effect of PDP on broiler growth performance, lesion score and serum oxidative activities compared to Eimeria-vaccinated broilers. A total of 600 one-day old broilers were randomly allocated into five dietary treatments with six replicates and 20 birds each. The five experimental treatments included 1) a control that broilers fed a corn soybean meal-based diet; 2) broilers vaccinated against coccidiosis fed a control diet; and 3-5) unvaccinated broilers fed PDP supplemented at 0.05, 0.10 or 0.20 g/kg on top of the control diet. Birds and feed in each pen were weighed weekly for average daily feed intake (ADFI) and average daily gain (ADG). At 7 and 14, five birds per pen were randomly selected to collect serum samples for quantification of oxidative status parameters. The study was conducted as a completely randomized design, and data were analyzed using SPSS by
a one-way ANOVA. P values less than 0.05 were considered as significant. During the whole 21d period, broilers inoculated with live cocci vaccine showed a lower (P<0.05) ADG compared to the control group, but all three doses of PDP supplemented group showed a similar ADG compared to control group. Vaccinated birds showed a worse FCR (P<0.05) compared to the control group and PDP at 0.10 or 0.20 g/kg. At d7 and d14, birds vaccinated against cocci had higher (P<0.05) levels of diamine oxidase, malondialdehyde, interleukin-3, and interferon γ in the serum, whereas supplementing PDP at 0.05, 0.10 or 0.20 g/kg decreased (P<0.05) these levels compared to vaccinated birds. In addition, supplementation of PDP at 0.10 and 0.20 g/kg further reduced (P<0.05) diamine oxidase and malondialdehyde level compared to the control. At d14, supplementation of PDP at 0.20 g/kg decreased interleukin-3 in the serum compared to the control. In conclusion, cocci vaccine decreased chicken growth performance and caused oxidative stress and inflammatory status, whereas PDP supplementation at 0.05 to 0.20 g/kg showed no negative effects on growth performance and serum parameters.

Key Words: phytochemical pentadecylphenol, cocci vaccines, broiler, growth performance, serum parameters


The present study was conducted to evaluate the anticoccidial activity of a commercially available phytogenic feed additive (PFA) on broiler chickens subjected to a cocci challenge. A complete randomized design was used to distribute 2,400 male newly-hatched Ross-308 broiler chickens into 6 treatments (10 replicates of 40 chickens each) using floor pens with new wood-shavings bedding. Treatments were 1) Negative Control (NC; non-challenged, no additives); 2) Positive Control (PC; challenged, no additives); 3) SAL (challenged + 60 ppm Salinomycin); 4) C-VAC (coccid vaccinated and challenged, no additives); 5) PFAA (challenged + 500 ppm BIOADD®, Grupo Nutec®, Mexico); 6) PFAB (coccid vaccinated and challenged + 500 ppm BIOADD®). A 5-phase mashed sorghum-soy feeding program (Pre-starter 0-10 d; Starter 10-21 d; Grower 21-28 d; Finisher I 28-42 d; Finisher II 42-49 d) was used in this experiment. C-VAC and PFAB treatments were vaccinated with a commercial live cocci vaccine (Coccivac®-B52, Merck Animal Health) on d 1. All treatments except the NC were orally challenged at d 12 with 1 mL of a cocktail containing a mixture of Eimeria acervulina (1x10⁶), Eimeria maxima (2x10⁵) and Eimeria tenella (2x10⁵). Salinomycin and PFA were supplemented to the corresponding dietary treatments from d 10 to the end of the trial (10-49 d). Performance and skin pigmentation data were subjected to one-way ANOVA, and when significant (P<0.05), Tukey’s test was used to compare means. Lesion score data were analyzed using the Kruskal–Wallis test. Overall average Johnson and Reid scores at 21 and 28 d (9 and 16 days-post challenge) were 0, 0.7, 0.3, 0.2, 0.6, 0.2 and 0, 0.5, 0, 0, 0.1, 0.1 for NC, PC, SAL, C-VAC, PFAA and PFAB respectively. After 49 d, the chickens supplemented with the PFA (PFAA and PFAB) were heavier (P<0.05) than the C-VAC, PC and NC treatments. Additionally, feed conversion ratio (FCR) was improved (P<0.05) in all treatments when compared to the PC. An average improvement of 14% was observed in FCR between both PFA treatments and the PC. Additionally, PFAB treatment improved 2.9% the FCR when compared to the SAL treatment. Skin pigmentation of the chickens after 49 d was reduced (P<0.05) in the PC compared to all other treatments, and no difference (P>0.05) was observed between the SAL and both PFA treated groups. In conclusion, the anticoccidial activity of the PFA showed to be as effective as Salinomycin controlling coccidiosis and sustaining growth in broiler chickens specially when used in conjunction with the cocci vaccine. Thus, the results of the present study suggest that the PFA BIOADD® could be used as an alternative to ionophore anticoccidials.

Key Words: Phytonic feed additive, broiler, performance, coccidiosis, anticoccidial

181 Fatty acid properties and breast myopathy occurrences in chickens fed Quantum Blueat increasing dietary inclusions. Reagan N. Cauble*, Elizabeth Greene, Barbara Mallmann, Sara Orlowski, Jason Apple, Carrie Walk, Mike Bedford, Sami Dridi, Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, 2AB Vista, Marlborough, United Kingdom.

Breast myopathies are a growing concern in broiler chickens. Recent studies have outlined woody breast (WB) characteristics including infiltration of fat cells, however, defining the WB etiology and identifying strategies to reduce its occurrence warrants intensive fundamental and applied research. The present study aimed to investigate the effect of increasing dietary phytase inclusion on WB occurrences and fatty acid (FA) profiles. Broilers (n = 576) were assigned randomly to 1 of 6 dietary treatments: 1) a positive control with adequate P and Ca levels (PC), 2) the PC diet with 0.3% Myo-inositol included (PC+MIO), 3) a negative control with lower than recommended P and Ca levels (NC), 4) NC diet with 500 FTU/kg phytase (NC+500), 5) NC with 1000 FTU/kg phytase (NC+1000), or 6) NC with 2000 FTU/kg phytase (NC+2000). Phytase was quantum blue (AB Vista, UK). Birds had ad libitum access to feed and water for 56 days and were processed at the University of Arkansas Pilot Processing Plant using a commercial inline system. WB and white striping scores were recorded from 0 (normal) to 3 (severe) in 0.5 increments and categorized by WB severity as follows: 0, normal (NORM); 0.5 to 1.5, moderate (MOD); and 2 to 3, severe (SEV). Breast sections were excised for FA analysis
Comparison of dried egg product vs chemical anticoccidial drugs administered to chickens during an enteritis caused by *Eimeria spp.* and *Clostridium perfringens*. Nannette Olmeda-Geniec*, Greg F. Mathis², Brett Lumpkins¹, Jeffery Escobar¹, Jordan M. Sand¹, ¹Nutritional Health, Elanco Animal Health, Aston, Pennsylvania, United States, ²Southern Poultry Research, Inc., Athens, Georgia, United States, ³AbE Discovery, Madison, Wisconsin, United States.

Coccidiosis is attributed to increase broilers susceptibility to Necrotic enteritis (NE) development, consequently control of *Eimeria* infection may lessen NE incidence and severity. Dried egg product (DEP) containing anti-IL-10 IgY neutralizing antibody appears to improve growth performance of broilers during experimental NE model. This study compares DEP at 287 U/TK against nicarbazin (Phibro Animal Health, Teaneck, NJ) and zoalene (Zoetis, Parsippany, NJ) on growth performance of coccidiosis vaccinated broilers followed with *Clostridium perfringens (Cp)* challenge. Cobb 500 males were housed 50 birds/pen for 42 d in 50 floor pens with buildup litter. Diets were administered in 3 feeding phases: Starter (d 0-14), Grower (d 14-28), and Finisher (d 28-42). DEP was provided 0 to 28 d, nicarbazin 0 to 37 d, and zoalene 0 to 42 d of study. A randomized complete block design was used to evaluate a non-infected, infected control, DEP (287 U/TK), nicarbazin (100 ppm) and zoalene (125 ppm) under experimental NE, which consisted of Coccivac® B52 (Merck, Kenilworth, NJ) spray vaccination on d of hatch, and 10⁸ cfu/bird/d of *Clostridium perfringens (Cp)* in feed on d 15, 16 and 17. At d 17, 4 birds were randomly selected from each pen for NE lesion scoring. Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), and production efficiency index (PEI) were determined for each feeding phase and overall (0-42 d). Statistical analyses were conducted using JMP (v.14.1, SAS Institute, Cary, NC), P <0.05. There were no differences (P >0.16) in growth performance during the Starter phase, Cp pre-challenge phase. Cp challenge during Grower phase reduced (P<0.05) performance and survival, and increased (P<0.0001) NE lesion scores compared to the uninfected group, indicating a successful NE challenge. All three additives reduced (P<0.001) NE lesions compared to the infected control. Birds fed DEP-supplemented diets exhibited 7-pt lower FCR and 9.3% greater survival (P<0.05) than those of the infected control; there were no differences (P>0.12) in performance amongst DEP, nicarbazin, but zoalene effected lower FCR (P<0.001) than the DEP treatment. In conclusion, supplementation of DEP significantly improved overall bird performance as compared with infected birds and showed no significant difference on BW, ADG and PEI vs anticoccidial drug during 42-d study. DEP could be used as an alternative to control coccidiosis and thus ameliorate the negative effects of NE in broiler production.

Key Words: IL-10 IgY neutralizing antibody, Coccidiosis, dried egg product, anticoccidial drug, Necrotic Enteritis
184 Assessing the effect of feeding Bacillus subtilis and mannan oligosaccharide on immune responses and enteric Lactobacilli and Bifidobacteria loads in broiler birds. Ragini Reddyvari*, Poultry Science, University of Georgia, Athens, Georgia, United States.

This study analyzed the effects of feeding Bacillus subtilis (B. subtilis) and mannan oligosaccharide (MOS) on immune response and enteric Lactobacilli and Bifidobacteria loads in broiler birds. Two hundred one-day-old Cobb500 broilers were randomly assigned to three treatments and one control group. Male day-old Ross 308 birds (312 in total) were reared from d0 to d35. Four treatments were: two controls without or with NE challenge (CtrlNC and CtrlNE respectively); two treatments of probiotic (Alterion 500 mg/kg) and antibiotic (Zn Bacitracin 4.4 mg/kg) supplements with NE challenge (ProNE and AntNE). Diets were formulated with wheat and soybean-meal. NE challenge commenced with E. coli (approx. 10^8 CFU/bird) per os at d9 and Clostridium perfringens EHE-NE18 (approx. 10^8 CFU/bird) per os at d9 and d14. At d16 and d20, Lactobacilli load in cecal and jejunal contents were significantly (P<0.05) higher than that in the T1, T2, and T3 groups when compared with that in the control group. At d17, Bifidobacteria load in cecal and jejunal contents were significantly (P<0.05) higher in T1, T2, and T3 groups when compared with that in the control group. At d20, Bifidobacteria load in jejunal contents of birds in the T1, T2, and T3 groups were not significantly different from that in the control group (P>0.05). At d17, IL-1β mRNA amounts were higher in T1, T2, and T3 groups treatment groups when compared with control (P>0.05). At d20, IL-1β mRNA amounts were not significantly different from that in the control group (P>0.05). At d17, IL-10 mRNA levels were lower in T1, T2, T3 when compared with that in the control group (P>0.05). At d20, IL-10 levels were lower in T1, T2, T3 when compared with control (P>0.05). Findings demonstrate that B. subtilis and MOS supplementation have significant effects on production parameters and enteric Lactobacilli and Bifidobacteria load in ceca.

Key Words: Bacillus subtilis, Mannan Oligosaccharide, real time PCR, Lactobacilli, Bifidobacteria


This study investigated the benefit of a Bacillus subtilis-derived probiotic (Bs 29784) in necrotic enteritis (NE) challenged broilers. Male day-old Ross 308 birds (312 in total) were reared from d0 to d35. Four treatments were: two controls without or with NE challenge (CtrlNC and CtrlNE respectively); two treatments of probiotic (Alterion 500 mg/kg) and antibiotic (Zn Bacitracin 4.4 mg/kg) supplements with NE challenge (ProNE and AntNE). Diets were formulated with wheat and soybean-meal. NE challenge commenced with Eimeria spp 1ml/bird per os at d9 and Clostridium perfringens EHE-NE18 (approx. 10^8 CFU/bird) per os at d9 and d14. At d15, samples for 16S next-generation sequencing (NGS) in caecal content and gene expression analysis from jejunal tissue were obtained on d16 and/or d35. ANOVA procedure of XLSTAT, Chi-square and Kruskal-Wallis tests were used for performance/gene expression, microbiome relative abundance and Alpha diversity comparison among multi-groups, respectively. NE challenge suppressed the performance (27% weight gain reduction, 11 points FCR increase at d16 (P<0.001), and 12% weight gain reduction, 5 points FCR increase at d35 (P<0.001) compared to CtrlNC. By d35, ProNE and AntNE treatments enabled higher weight gain (approx. 4% (P< 0.033) and 9% (P<0.001), respectively, than CtrlNE. Compared with CtrlNE, and contrary to AntNE, ProNE treatment exhibited...
upregulation of genes coding tight junctions proteins (CLDN1, P<0.01, JAM2, P<0.001), cytokines (IL12, P<0.05, IFNγ P<0.001, TGFβ, P<0.01) and Toll-like receptors (TLR5, P<0.05, TLR21, P<0.05) suggesting enhanced immunity and intestinal integrity. Besides, 16S NGS analysis of caecal microbiota at d16 showed a decreased (P<0.05) alpha diversity in challenged groups. Principal component analysis (PCA) of OTUs abundance revealed that ProNE and AntNE grouped closely while CtrlNC and CtrlNE grouped separately, indicating the similarity of OTUs between ProNE and AntNE birds that were however different from both CtrlNC and CtrlNE birds. Microbiota analysis also revealed an increase (P<0.001) of genera Faecalibacterium and Butyricicoccus and a decrease of genera Lactobacillus and Bacteroides, in addition to a decrease of Firmicutes to Bacteroidetes ratio in ProNE and AntNE groups compared with CtrlNC group. It is concluded that Bs 29784 may enable improved health of broiler chickens under NE conditions.

**Key Words:** Bacillus subtilis, Clostridium perfringens, microbial diversity, intestinal barrier, inflammation

### 186 Using yucca and/or phytogenics on alleviating negative effects of necrotic enteritis in broilers. Lan Zheng*,1, Johel Bielke2, Shelby Ramirez2, Chasity M. Pender1, Antonia Tacconi1, G. R. Murugesan1, Lisa Bielke2, 1BIOMIN North America, Overland Park, Kansas, United States, 2The Ohio State University, Wooster, Ohio, United States, 3BIOMIN Holding GmbH, Getzersdorf, Austria.

Increased antibiotic-free broiler production has driven the demand for non-antibiotic solutions for disease challenges including necrotic enteritis. The objective of this experiment was to evaluate the effects of a novel phytogenic feed additive (PEPY, Digestarom® PEP-Y, BIOMIN America Inc.) on growth performance of broilers using a necrotic enteritis (NE) challenge model compared to three commercially available PFA (phytogenic feed additive) products. A total of 800 day-old Cobb-500 broilers were randomly placed to 1 of 5 dietary treatments (8 replicate pens/treatment, 20 birds/pen) and fed diets over 3 phases. Dietary treatments consisted of: 1) CON, non-challenged treatment; 2) YUC1, challenged + PFA composed of Yucca Schidigera and Quillaja Saponaria; 3) YUC2, challenged + PFA derived from Yucca Schidigera; 4) EO, challenged + PFA containing a mixture of essential oils; and 5) PEY, challenged + Digestarom® PEP-Y. On d 15, all challenged birds were individually inoculated with 66,250 oocysts of *Eimeria maxima*. On d 20, birds in the challenged treatments additionally received 1 x 10^7 CFU/mL of *Clostridium perfringens*. BW and feed disappearance were measured at the end of each phase and mortality was recorded. Data were analyzed using GLIMMIX procedure of SAS. During d 0 to 14, no differences were found among the treatments. During d 0 to 21, BWG was decreased (P < 0.05), and FCR was increased (P < 0.05) in all challenged treatments compared with those from CON. Birds fed PEY tended to have higher (P = 0.073) BWG than those fed EO and showed numerical improvement compared with YUC2. During the entire 42 d, all challenged treatments had lower (P < 0.05) BW compared with CON. No differences were observed in FCR among the treatments, whereas birds fed PEY had numerically lower FCR compared to those fed YUC2. All challenged treatments had higher NE mortality compared with CON, whereas PEY showed a numerical reduction in non-NE mortality compared with YUC1. These results suggest the addition of PEY in broiler feed may have resulted in better livability and performance in comparison to the other three PFA products tested.

**Key Words:** broilers, coccidiosis, growth performance, necrotic enteritis, phytogenic feed additives

### 187 Effects of Berberine on humoral immune response, hematological parameters, and lymphoid organ weights during aflatoxicosis and ochratoxicosis in broilers. Pouyan Malekiniazhad*,1,2, Laura Ellesta^2, Nazar Afzali, Seyed Homayoun Farhangfar, Arash Omidib, Abbas Mohammadi, *Poultry Science, University of Georgia, Athens, Georgia, United States, †Animal Sciences, University of Birjand, Birjand, Iran (the Islamic Republic of), ‡Animal Health Management, University of Shiraz, Shiraz, Iran (the Islamic Republic of), †Plant Pathology, University of Birjand, Birjand, Iran (the Islamic Republic of).

Mycotoxin-contaminated feed causes mortality, suppression of the immune system, reduced growth rates, and losses in feed conversion ratio. Berberine hydrochloride (berberine; BBR), a natural plant alkaloid derived from Chinese medicine, is characterized by diverse pharmacological effects. This study is designed to evaluate effects of BBR on humoral immune response, lymphoid organs (thymus, spleen, and bursa of Fabricius), and hematological parameters of broilers fed diets contaminated with Aflatoxin B1 (AFB) and Ochratoxin A (OCT). A 42-day floor pen trial was performed with 288 Ross 308 broilers. A randomized design, with 4 replicates of 8 birds each, was conducted with the following 9 treatments: (1) negative control diet with no additives (NC); (2) NC + 2 ppm AFB (positive control AFB; PCAFB); (3) NC + 2 ppm OCT (positive control OCT; PCOCT); (4) PCAFB + 200 mg/kg BBR; (5) PCAFB + 400 mg/kg BBR; (6) PCAFB + 600 mg/kg BBR; (7) PCOCT + 200 mg/kg BBR; (8) PCOCT + 400 mg/kg BBR; and (9) PCOCT + 600 mg/kg BBR. To evaluate the humoral immunity, sheep red blood cells (SRBC) were injected into the blood through the brachial vein of broilers in each group at days 28 and 35, and antibody titers were measured 7 days after each injection. At the end of the experiment, two chicks weighing close to the average pen weight (mean±1 SD) were selected at random from each pen (8 chicks/treatment). From each bird, blood samples were taken to determine hematological parameters [white blood cells (WBC), neutrophil (NEU), and lymphocyte (LYM)], after which thymus, spleen, and bursa of Fabricius weights were recorded. Data were subjected to statistical analysis using the general linear
model procedure of SAS. Parameters showing significant differences in the one-way analysis of variance were compared using Tukey’s general linear model test. All the statements of significance were based on the 0.05 probability level. Compared to NC, PCAFB and PCOCT treatments reduced (P<0.05) the number of WBC, LYM, and NEU, and the weights (% body weight) of thymus and bursa of Fabricius. Levels of immunoglobulins and antibody titers against the SRBC challenge also decreased (P<0.05) compared to NC. In contrast, compared to PCAFB, adding 600 mg/kg of BBR to contaminated diets increased (P<0.05) WBC and antibody titers against the SRBC challenge. Adding 600 mg/kg of BBR to the PCOCT diet significantly increased the level of IgG compared to PCOCT (P<0.05). It can be concluded that BBR may improve immune parameters in aflatoxicosis and ochratoxicosis.

Key Words: Aflatoxin, Berberine, hematology, immunoglobulin, Ochratoxin

188 Effect of n-3 FA and Vit D3 in keel bone properties and Ca/P transporter profile in aviary Lohmann browns. Dima L. White*1, Darrin M. Karcher2, Prafulla Regmi3, Woo Kim1, 1Poultry Science, University of Georgia, Athens, Georgia, United States, 2Animal Science Department, Purdue University, West Lafayette, Indiana, United States, 3Prestage Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, United States.

Keel bone fractures & deformations constitute the most pressing welfare concern in laying hens at present. Fracture prevalence of 80% has been observed in cage-free systems throughout the European Union with highest incidences occurring around 30 wks of age. 200 million laying hens are at risk of keel fractures with the U.S. as the egg industry adopts cage-free systems in the future. Alternative nutrition strategies such as inclusion of omega-3 polyunsaturated fatty acids (n-3 fatty acids) and 25 hydroxyvitamin D3 (25-(OH)D3) in the diet can potentially mitigate keel bone issues in laying hens housed in cage-free aviary. We hypothesized that birds supplemented with n-3 fatty acids & 25-(OH)D3 in the diet would have structurally superior keel and less fractures versus control birds. The objectives were to identify strategies to augment laying hen keel bone properties, and to determine the relationship between the intestinal Ca & P transporters and keel bone properties in laying hens. A 52wk trial was conducted with alternative feed additives to single brown-egg strain (Lohmann Brown-Lite) raised in multiple house symptoms. Dietary treatments were; 1) Aviary control (AC), 2) n-3 fatty acid diet-linolenic:alpha-linolenic acid ratio 0.6:1 (flaxseed source of n-3), 3) Polyunsaturated fatty acids (PUFA) diet- tuna fish oil; EPA and DHA (n-6:n-3 = 0.6:1), and 4) Vitamin D diet-25-(OH)D3 at 2,760 IU/kg. Keels were collected at 18, 32 & 52 wks of age and scanned by a Micro-CT to observe the bones structure and composition. Jejunum samples were collected to determine transporter gene expressions. Data were subjected to a one-way ANOVA using the GLM procedure, with means deemed significant at P<0.05. Results indicated no significant observations among any treatments for keel bone scanning analysis, however, at 32wks the 25-(OH)D3 was numerically higher for the total bone volume (20.6mm³), percentage bone volume (1.224%), bone mineral density (0.019g/cm³), and bone mineral content (0.025g) compared to AC. This indicates that possibly 25-(OH)D3 created a larger keel bone structure which means more space for mineral deposition. Transport gene expression results at 18wks observed significant increase in calcium binding protein (calbindin-D 28k) for n-3 fatty acid treatment, and at 32wks a significant increase in calbindin-D 28k & regulation of phosphate levels (sodium co-transporter) for PUFA & 25-(OH)D3 treatments compared to other treatments. At 52wks, a significant increase in calbindin-D 28k with PUFA treatment being higher than AC treatment. In conclusion, 25-(OH)D3 & PUFA can potentially improve keel bone structure and calcium binding protein along with regulation of phosphate levels.

Key Words: keel bone, cage-free, laying hens, transport gene, bone 3D structure

189 Effect of dietary phosvitin on shell and bone quality in end-of-cycle laying hens. Daniella Batres*1, Jiangping Wu, Douglas R. Korver, Agricultural, Life and Environmental Sciences, University of Alberta, Edmonton, Alberta, Canada.

Laying hens mobilize calcium from both medullary and structural bone, which can result in bone weakness, fractures and osteoporosis. In rat models, the egg yolk phosphoprotein phosvitin may increase dietary Ca absorption and reduce osteoclastic breakdown of bone. The effects of dietary phosvitin on shell and bone quality of laying hens (90 to 94 wk of age) were studied. Prior to the experiment, birds were housed individually in cages (2,064 cm²/bird) and acclimatized for 6 weeks on a positive control diet (PC; 3.6% Ca, 0.33% available P, with other nutrients according to the Lohmann LSL-Lite management guide). Birds (n = 8) were euthanized for baseline bone quality measurements. The hens were fed the experimental diets for 28 days. The treatments were: PC; negative control (NC) diet with 25% less Ca; either phosvitin (37.4% purity), dephosphorylated phosvitin (39.5% purity) or phosvitin peptides (39.8% purity), each fed at a 1% or 0.01% of the diet (8 treatments; n= 20 birds/treatment). The extreme Ca reduction was intended to induce a deficiency, in order to test our hypothesis, but was limited to four weeks duration to protect bird welfare. Body weight and shell quality were recorded at 90 and 94 wk of age. Egg production and feed consumption were recorded daily. At 94 wk of age, all birds were euthanized, and shell and femur bone quality (i.e. ash and breaking strength) were measured. Data were analyzed using a one-way ANOVA with means separated using Tukey’s test (SAS 9.4; SAS Institute Inc, Cary, NC); significance was set at p ≤ 0.05. There were no treatment effects on femur breaking strength, bone ash, shell
thickening, specific gravity, shell breaking strength, shell weights nor egg weights. Final body weight and feed conversion ratio (FCR; g of feed/g of egg weight) were not affected. Egg production of birds fed 1% dephosphorylated phosvitin was lower than the PC birds (82.75% vs 96.76% respectively, $\pm$ 0.063; $p = 0.014$), but there were no other treatment effects. The effect of phosvitin and its derivatives could not be assessed due to a lack of an NC effect on shell quality, bone traits and performance. Ca in the NC diet was not clinically deficient, since the laying hens were able to compensate for the 25% reduction in dietary Ca, at least in the short term, since shell and bone quality were unaffected. A longer experimental duration may yield different results, however, this would be at the expense of hen welfare. The lack of effect on bone mineralization was likely due to an increase in intestinal Ca absorption and decreased renal Ca excretion in order to maintain shell and bone quality.

**Key Words:** phosvitin, laying hens, calcium, bone health, shell quality

**190 Production of omega-3 enriched eggs with dietary water-soluble flaxseed oil.** Sang Hyeok Lee, Yoo Bhin Kim, Da-Hye Kim, Dong Won Lee, Hong-Gu Lee, Kyung-Woo Lee, Konkuk University, Seoul, Korea (the Republic of), Haitnim Bio, Icheon-si, Korea (the Republic of), Konkuk University, Seoul, Korea (the Republic of), Konkuk University, Seoul, Korea (the Republic of).

This present study was designed to produce omega-3 enriched eggs using water-soluble flaxseed oil (SFO) in the diets of laying hen chickens. Powdered SFO were manufactured from flaxseed via a patent solubilized process to remove antinutrients present in flaxseed and to make a water-soluble powdered form as food additive for human consumption. It was expected that dietary SFO would affect laying performance, egg quality, serum parameters and fatty acid composition of egg yolk in laying hens. A total of 210 Hy-Line Brown laying hens of 52 weeks old were randomly allotted into one of five dietary treatments: no-added control diet and diets containing SFO at the levels of 0.2%, 0.4%, 0.6%, and 0.8% for four weeks. Data were analyzed by ANOVA using the GLM procedures of SAS. Orthogonal polynomial contrasts were used to determine linear and quadratic effects of graded added SFO levels on the variables. Significances were preset at $P < 0.05$. Dietary SFO did not affect laying performance including egg production, feed intake, egg weight and feed conversion ratio. None of egg qualities was affected by dietary treatments. Upon storage of eggs at room temperature, increasing dietary SFO levels linearly increased ($P < 0.05$) the pH and malondialdehyde contents of egg yolk. A significant increase ($P < 0.05$) in omega-3 fatty acids, especially docosahexaenoic acid, was detected in egg yolks from hens fed the diets containing dietary SFO at 2 and 4 weeks. An omega-3:omega-6 ratio was increased ($P < 0.05$) with increasing dietary SFO supplementation. In conclusion, the present study suggests that dietary SFO did not affect laying performance but increased the deposition of docosahexaenoic acid in egg yolk.

**Key Words:** water-soluble flaxseed oil, laying hen, laying performance, egg storage, fatty acid composition


The effects of substitution of graded levels of processed yam peel (PYPM) for maize with multi-enzymes cocktail + yeast (MECY) supplementation in the diet of Japanese quails (Coturnix coturnix japonica) on laying performance and egg quality traits were studied in a 10-week experiment. The yam peels were processed by soaking in water for 72 hours, sun-dried for 48 hours and thereafter milled using a hammer mill to obtain the PYPM. A total of 240, seven-weeks old Japanese quails were distributed in a completely randomize design into four dietary treatments and five replicates of 12 quails each. Diet 1 served as the control diet without PYPM while diet 2, 3 and 4 contained 25, 50 and 75% PYPM with MECY supplementation (1g per kg diet), respectively. Each gram of MECY contained a mixture of phytase (2500 FTU), cellulase (10,000 IU), $\beta$-glucanase (200 IU), xylanase (10,000 IU) and yeast (Saccharomyces cerevisiae, 1x10^7 cfu). Performance indices were evaluated by weekly quantification of feed leftovers and average egg weight to determine hen-day egg production (HDP, %), feed intake (FI, g day$^{-1}$), egg weight (EW, g), egg mass (EM, g day$^{-1}$) and feed conversion per egg mass (FCEM, g of egg g of diet$^{-1}$). Egg quality traits were determined by measuring EW, egg width (mm), egg height (mm), egg shape index (%), albumen height (mm), Haugh unit (HU), yolk weight (YW, g), eggshell weight (ESW, g), albumen weight (AW, g), eggshell thickness (EST, mm) and yolk index. All data were analyzed using one-way analysis of variance of the GLM procedure of SAS software package and differences among treatment means were determined using Tukey test. Statement of statistical significance were declared at 5% probability ($p<0.05$). The results indicated that no significant ($p>0.05$) difference was recorded on feed intake, EW and HDP; however, improved ($p<0.05$) EM and FCEM were observed with increasing levels of MECY supplemented PYPM. The internal and external egg quality measurements except for ESW and EST were not affected ($p>0.05$) by inclusion of PYPM with MECY supplementation. The ESW and EST in quails fed 50 and 75% MECY supplemented PYPM diet were similar.
(p>0.05) to those fed control diet but higher (p>0.05) than the group fed 25% PYPM diet. In conclusion, the combine use of multienzyme cocktail containing phytase, cellulose, β-glucanase, xylanase, and yeast (Saccharomyces cerevisiae) can enhance the utilization of PYPM in replacing up to 75% of maize in diets of laying Japanese quails without adverse effect on performance and egg quality traits.

**Key Words:** Quails, Laying performance, Egg quality, Multi-enzymes, Yeast

192 Not Presented.

193 **Methionine activates JAK2/STAT5 by regulating prolactin to promote crop milk protein synthesis during lactation of domestic pigeons (Columba livia).** Meng-jie Chen**, Xiu-qi Wang, Hui-chao Yan, Chun-qi Gao, College of Animal Science, South China Agricultural University/Guangdong Provincial Key Laboratory of Animal Nutrition Control, Guangzhou, China.

Unlike other poultry species, pigeons are altricial birds, and young pigeons (squabs) must rely on their parents to feed them crop milk for nourishment in the early stage of growth. Protein is the main ingredient in crop milk; however, its regulation mechanism remains unclear. In this study, three experiments were conducted to investigate the possible underlying mechanism of crop milk protein synthesis, and its nutritional interventions. In experiment 1, 6 pairs in the lactation period and 6 pairs in the non-breeding period of breeding pigeons were used to compare the protein expression changes in crop tissues of pigeon by isobaric tagging for relative and absolute quantification (iTRAQ). In experiment 2, 40 pairs of breeding pigeons were randomly distributed to 4 treatments. From the 1st day of lactation, the breeding pigeons were respectively injected with 0 (Control, physiological saline), 0.5 mg/kg, 1.0 mg/kg, and 2.0 mg/kg body weight (BW)/day bromocriptine mesylate, a prolactin (PRL)-specific inhibitor, through wing vein. On the 8th day, the pigeons were euthanized to collect the samples of serum and crop tissue. In experiment 3, 324 pairs of breeding pigeons were randomly assigned to 9 treatments for 45 days, including a control diet (CON; basal diet), four DL-Methionine (Met) groups (0.15, 0.30, 0.45, and 0.60%) and four DL-Methionine-DL-Methionine (Met-Met) groups (0.15, 0.30, 0.45, and 0.60%). On the 21st day, 6 pairs of pigeons from per group were euthanized to collect the samples of serum and crop tissue. The t-test or Duncan's multiple range tests of SAS 9.2 were used to determine the differences between two groups or more groups, respectively. The iTRAQ analysis found that the janus activated kinase (JAK) / signal transducers and activators of transcription (STAT) pathway was up-regulated in the breeding pigeons during lactation compared to non-breeding (P < 0.05). The proteins expression of PRL receptor (PRLR)/JAK2/STAT5 pathway were increased during lactation (P < 0.05). The serum PRL, the PRLR/JAK2/STAT5 pathway, the crop milk protein synthesis, and the squab growth performance were inhibited by bromocriptine mesylate injection (P < 0.05). In addition, dietary supplementation with 0.30% Met or Met-Met (especially 0.30% Met-Met), increased serum PRL and PRLR/JAK2/STAT5 activity (P < 0.05), improved the crop milk protein synthesis and the squabs growth performance (P < 0.05). In conclusion, we found that the PRL-induced PRLR/JAK2/STAT5 pathway plays a vital role in crop milk protein synthesis, and 0.30% Met-Met is superior to Met to promote squab growth. Funding for this project was provided by the Natural Science Foundation of Guangdong Province, China.

**Key Words:** Pigeon, crop milk, DL-methionine, prolactin, PRLR/JAK2/STAT5 signaling pathway
Crude protein feed content on productive performance and characteristics of eggs from second-cycle laying hens in Mexico. Fernando Sánchez-Morales\textsuperscript{1,2}, Jorge L. Cruz-Zúñiga\textsuperscript{1}, Diego Zárate-Contreras\textsuperscript{2}, Arturo Pro-Martínez\textsuperscript{2}, Marco A. Rivas-Jacobo\textsuperscript{2}, Fernando González-Cerón\textsuperscript{1}, \textsuperscript{1}Autonomous University of San Luis Potosi, San Luis Potosi, San Luis Potosi, Mexico, \textsuperscript{2}Department of Animal Science, Chapingo Autonomous University, Texcoco, State of Mexico, Mexico, \textsuperscript{3}Livestock Program, College of Postgraduates Campus Montecillo, Texcoco, State of Mexico, Mexico.

The aim of this study was to evaluate the effect of commercial feed formulated for laying hens, with two levels (16 and 18\% of crude protein (CP)), on productive performance and characteristics of eggs from second-cycle laying hens in Mexico. Four hundred and twenty two Leghorn-type laying hens on their second cycle of production (100 wk of age) were randomly allocated into two treatments (211 birds per treatment): 16\% PC, commercial feed with 16\% of PC; and 18\% PC, commercial feed with 18\% of PC. Birds were allocated in two level pyramidal batteries (two hens per cage) under a 16 h light:8 h dark regime. Daily feed intake was 115 g bird\textsuperscript{1} of feed. The evaluation period lasted 52 d. Productive performance variables were: egg production (EP, \%), egg weight (EW, g), egg mass (EM, g hen\textsuperscript{-1} d\textsuperscript{-1}), and feed conversion ratio (FCR). Based on a sample of 64 eggs per treatment, collected randomly during the last eight d of the study (eight eggs d\textsuperscript{-1} treatment\textsuperscript{-1}), additional egg variables were recorded in each egg: length (L, cm), width (W, cm), shape index (SI, \%), eggshell percentage (ESP, \%), albumen percentage (AP, \%), and yolk percentage (YP, \%). Data were analyzed by ANOVA with GLM procedure of SAS. For performance variables, every day was considered a replicate of the treatment. In contrast, for egg variables, every sampled egg constituted a replicate. Flock in 18\% PC treatment showed better values (P < 0.05) on EP, EW, EM, and FCR (91.4±0.3\%, 63.0±0.1 g, 57.6±0.2 g hen\textsuperscript{-1} d\textsuperscript{-1}, and 1.998±0.009, respectively) comparing to 16\% PC birds (89.6±0.3\%, 61.9±0.1 g, 55.5±0.2 g hen\textsuperscript{-1} d\textsuperscript{-1}, and 2.075±0.009). On the other hand, eggs from 18\% PC birds were longer (6.001±0.035 cm) and wider (4.465±0.019 cm) than those from 16\% PC hens (5.833±0.035 cm and 4.387±0.019 cm, respectively). No differences (P > 0.05) were detected between treatments on SI (16\% PC: 75.3±0.4\%; 18\% PC: 74.5±0.4\%) and YP (16\% PC: 28.8±0.3\%; 18\% PC: 28.2±0.3\%). However, eggs from 18\% PC birds had higher (P < 0.05) AP (61.3±0.4\%) and lower (P < 0.05) ESP (10.5±0.2\%) than eggs from hens in 16\% PC treatment (59.6±0.4\% and 11.6±0.2\%, respectively). In conclusion, available commercial feed with 18\% PC for laying hens in Mexico, improves productive performance of birds on their second cycle of production and some characteristics of the produced eggs, such as weight, size and proportion of albumen.

**Key Words:** laying hens, protein, productive performance, egg

Visceral mass and allometry of metabolic size in laying pullet. Rony Riveros Lizana\textsuperscript{4,5}, Freddy Horna Morillo\textsuperscript{1}, Carolina Cardoso Nagib Nascimento\textsuperscript{1}, Matheus D. Reis\textsuperscript{2}, Bruno Balbino Leme\textsuperscript{1}, Nilva Sakomura\textsuperscript{1}, 'Animal Science, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil, 'Animal Science, UNESP, Jaboticabal, Sao Paulo, Brazil.

The description of the metabolic size (BW\textsuperscript{b}) from the basal metabolic heat (FHP=a×BW\textsuperscript{b}) and their allometric relationship with the visceral mass, could be an important step to understand the poultry energy expenditure. The objective was to estimate and compare the allometric coefficient of the metabolically active organs and describe them by the basal energy expenditure in growing pullets. One hundred forty-four Lohmann LSL pullets of one to seven weeks-old were used, housed in a climatized farmhouse. Weekly, a group of twenty birds with the same body weight (BW), were distributed randomly in two calorimetric chambers to measure the oxygen consumption (VO\textsubscript{2} L/d) and carbon dioxide production (VCO\textsubscript{2} L/d). It was considered three adaptation days to chambers and one day (24h) for measurement of gas exchange under fasting. The VCO\textsubscript{2} and VO\textsubscript{2} were used to calculate the respiratory quotient (RQ), and the heat production (HP) by the Brower equation HP(kJ/d)=16.18×VO\textsubscript{2}+5.02×VCO\textsubscript{2}. The obtained FHP was the value of HP at the minimum plateau and an RQ~0.7 (fasting state). At the final of the fasting period, birds were slaughtered, and visceral organ weight (gut, liver, heart) and breast were registered. The adjustment of the allometric equation was made by using the BW as the independent variable and the FHP, and weight of the gut, liver, heart, and breast as dependent variables. The data of each dependent variable were transformed into natural logarithm (ln), regressed, according to ln(BW). All statistical procedures were performed in SAS software. The metabolic size description from FHP was obtained the allometric exponent of \(b_{\text{FHP}}=0.714±0.035\) (P<0.001). While was showed that the allometry of the liver (\(b=0.752±0.015\), P<0.001) and gut (\(b=0.777±0.014\), P<0.001) was statistically similar (P<0.05), and different to the breast (\(b=1.171\), P<0.001) and to the \(b_{\text{FHP}}\). On the other hand, the heart allometry (\(b=0.708±0.02\), P<0.001) was close to \(b_{\text{FHP}}\) (P<0.05). Liver and gut metabolic activity is more related to the feeding process, consequently with the heat increment of the feed, differing of the FHP. Also, the breast due to a gain protein mass is colinear with body size, which differs from the metabolic size description. The allometry of heart mass is similar to the \(b_{\text{FHP}}\) since this organ has a continuous metabolic activity to maintain the homeostasis (nutrient
transport or gas exchange) and is not influenced by the feeding process, being one of the main components, could have a significant contribution to the basal metabolism. By describing the heart growth to describe the metabolic size, is an important step to support future research to measure the contribution of this organ on basal metabolism of laying pullets.

**Key Words:** energy metabolism, indirect calorimetry, poultry, fasting heat production, organ mass

197 Effects of dietary supplementation with fermented feedstuffs on the performance, egg quality and intestinal health of laying hens. Shi-guang Jiang68*, Chen Zhong, Xiu-qi Wang, Chun-qi Gao, Department of Animal Science, South China Agricultural University, Guangzhou, China.

A complete ban on the use of antibiotics as growth promoter will certainly affect the production of commercial laying hens, which is urgent to develop an effective antibiotic alternative. The objective of this study was to explore the effects of dietary supplementation with fermented soybean meal (FSM) and fermented cottonseed-coconut mixed meal (FCMM, cottonseed meal: coconut meal=1: 1) on the laying performance, egg quality, intestinal development, and follicle development of laying hens. A total of 1,008 of 54-week-old laying hens were randomly divided into 7 treatments, with 6 replicates per treatment. The control group was fed with a basic diet, and in the other 6 groups the soybean meal in the basic diet was replaced with 2%, 4%, and 8% FSM or FCMM, respectively. The experiment lasted 12 weeks. Data were analyzed with one-way ANOVA using the PROC-GLM procedure of SAS 9.2 software. Results showed that dietary supplementation with 2%-4% FSM significantly increased the laying production, and egg quality (P<0.05). Supplementation of 4%-8% FCMM significantly increased the egg yolk color (P<0.05). Supplementation of 4% FSM or FCMM significantly increased the relative weight, trans-epithelial electrical resistance, villi height, and crypt depth of jejunum, as well as the protein expression of zonula occludens-1 (ZO-1), Occludin and Claudin 1 in the jejunum (P<0.05). In addition, fed with 2%-4% FSM or FCMM significantly increased the ovarian relative weight, the number of small yellow follicles, and large white follicles (P<0.05). However, the 8% FCMM supplementation significantly decreased the laying production performance, the apparent metabolic rate of nutrients, and intestinal barrier function (P<0.05). The main effect analysis showed that FSM has a better effect than FCMM in improving the laying performance and egg quality of laying hens (P<0.05). In conclusion, dietary FSM and FCMM supplementation improved laying performance, egg quality, the apparent metabolic rate of nutrients, intestinal health, and follicle development of laying hens, especially for the supplementation of 4% FSM.

**Key Words:** Laying hen, Fermented feed, Laying performance, Egg quality, Intestinal health

198 The effect of partial replacement of limestone with gypsum in layer diets on hen productivity, egg quality, ammonia emissions and ammonia emission intensity of egg production. Matt Oryschak*, 1, Eduardo Beltranena1, 2, Alberta Agriculture and Forestry, Edmonton, Alberta, Canada, 2Alberta Agriculture and Forestry, Edmonton, Alberta, Canada, 2Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, Alberta, Canada.

Ammonia (NH3) emitted from poultry excreta in conventional confinement housing systems can result in concerns related to occupational hygiene for workers, welfare of laying hens and the environment. Because NH3 emission rate is influenced by pH, it may be possible to reduce NH3 emissions through dietary interventions designed to increase the acid-forming potential of the diet. The objective of this study therefore was to study the effectiveness of stepwise substitution of gypsum for limestone in hen diets at reducing NH3 emissions from hen excreta. Laying hens (n=128; Lohmann LSL-Lite) housed 4 per cage in a conventional layer battery served as the test system for this study. Hens (32 – 48 wk of age) received one of 4 nutritionally-complete layer diets where the ratio of supplemental Ca supplied by limestone versus gypsum was 100:0, 75:25, 50:50, in a randomized complete block design with 8 replicate cages per treatment. All diets contained titanium dioxide as an indigestible and stable marker to facilitate N mass balance calculations. Within each of four 4-wk phases, feed consumption, egg production, objective egg quality were measured. Feed and excreta sampled from each test cage in each 4 wk phase were analyzed for major N chemical species. A mass balance approach (N emissions = intake N – egg mass N – non-volatile excreta N) was used to calculate NH3 emissions and NH3 emission intensity. Data were analyzed as generalized linear mixed models using the GLIMMIX procedure of SAS (v9.4). Models included the main effect of limestone Ca:gypsum Ca (100:0, 75:25, 66:33, 50:50) and block as a random term; and with the exception of lay percentage, specified a normal distribution. Data were further analysed for linear-patterned responses to gypsum inclusion. As gypsum inclusion increased, there was a slight (<6%) but significant decline in egg mass:feed (P<0.01), suggesting that dietary nutrient density was not maintained across all dietary regimens. Other than slight linear declines in eggshell thickness (P<0.05) and specific gravity (P<0.01), increasing gypsum inclusion had no adverse effect on objective measures of egg quality. As gypsum inclusion in the diet increased, the pH of layer excreta declined linearly (P<0.01) and the amount of total N retained in excreta at 4, 8, 12 and 16 wk post-excretion linearly increased (P<0.01). The calculated NH3 emission intensity linearly decreased (P<0.01) with increasing gypsum inclusion, such that a 37% difference was observed between the 100:0 and 50:50 dietary regimens.
Ammonia (NH₃) emissions in poultry production are a consequence of the enzymatic decomposition of uric acid, which is the major form of excreted metabolic N. Metabolic N excretion is presumably influenced by the concentration and AA profile of digestible crude protein (DCP) in the diet. The objective of this study was to examine the relationship between increasing DCP content over a practical range and NH₃ emissions. Laying hens (n=160; Lohmann LSL-Lite) housed 4 per cage in a conventional layer battery served as the test system for this study. Test cages were fed one of five nutritionally-complete, wheat-based diets formulated to exhibit either linear or quadratic responses to increasing DCP content in wheat-based hen diets on hen productivity, egg quality and ammonia emission intensity of egg production. Matt Oryschak*, Eduardo Beltranena1, 2, ‘Alberta Agriculture and Forestry, Edmonton, Alberta, Canada, ‘Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, Alberta, Canada.

Ammonia (NH₃) emissions from each test cage in each 4 wk phase of production, objective egg quality were measured. Feed and experiment with 8 replicate cages per treatment. All diets contained 0.25% titanium dioxide as an indigestible and stable marker to facilitate N mass balance calculations. Within each of four 4-wk phases, feed consumption, egg production, objective egg quality were measured. Feed and excreta sampled from each test cage in each 4 wk phase were analyzed for major N chemical species. A mass balance approach (N emissions = intake N – egg mass N – non-volatile excreta N) was used to calculate NH₃ emissions and NH₃ emission intensity. Data were analyzed as generalized linear mixed models using the GLIMMIX procedure of SAS (v9.4). Models included the main effect of DCP content and block as a random term. With the exception of lay percent models assumed a normal distribution. Data were further analysed for linear and quadratic responses to dietary DCP content. There were slight (<1%) significant differences (P<0.05) among individual treatments for feed disappearance, average egg wt, proportional shell wt, and eggshell thickness that did not exhibit either linear or quadratic responses to increasing dietary DCP content. Most other hen productivity or egg quality metrics were unaffected by dietary treatment (P>0.10), suggesting general uniformity among all dietary treatments insofar as essential nutrient density in proportion to AME. Initial levels of total N and uric acid N in excreta linearly increased (P<0.01) with increasing dietary DCP. There was a u-shaped quadratic response (P<0.01) of NH₃ emission intensity (g NH₃/doz eggs) to increasing DCP, with the minimum value obtained between the 14.75% and 15.25% treatment. The quadratic response appears to be the result of a greater proportion of excreted N from hens being in volatile form for the lower DCP treatments. The results of the present study suggest that to limit excess NH₃ emissions, wheat-based laying hen diets formulated to meet optimal digestible AA recommendations for egg production should not exceed 15% DCP.

Key Words: Laying hens, Ammonia, Gypsum, Sustainability, Environment

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200 Inoculation in ovo of putrescine on egg characteristics and hatchability rate of eggs from broiler breeders. Isabella C. Dias*, Katiucia C. Sonalio, Filipe A. Moreno, Rosiane Araujo, Marley C. Santos, Chayane Rocha, Alex Maiorka, Federal University of Paraná, Curitiba, Paraná, Brazil.

The objective of this study was to evaluate the effects of solutions containing increasing levels of putrescine inoculated “in ovo” in eggs from broiler breeders. A total of 640 eggs from 34 week-old Ross® broiler breeder were used. All eggs went through the same handling, disinfection, storage and incubation periods. The experimental design was completely randomized, with 5 treatments and 8 replicates of 16 eggs each. The treatments were: 1) without inoculation (control group), 2) 0.015% putrescine, 3) 0.030% putrescine, 4) 0.060% putrescine and 5) 0.090% putrescine. At the 17th day of incubation, the eggs (except the control group) were sanitized with alcohol (70%) and 0.5 ml of putrescine was inoculated in the amniotic fluid. The analyzed variables included egg weight before incubation, chicken weight, ratio between egg weight and chicken weight (REW x RCW) and hatchability rate. The data were submitted to Shapiro-Wilk normality and Bartlett’s homogeneity. Subsequently, ANOVA was performed, and when significative, a binomial regression was conducted. Egg weight, chicken weight and REWxRCW showed no statistical difference between putrescine solutions (P>0.05). However, a statistical difference was observed for hatchability rate (P<0.01), showing that eggs without inoculation had better hatchability, as increasing levels of putrescine led to a linear decrease on hatchability rates (88.11% in control group; 77.47% for T2; 72.17% for T3; 74.39% for T4, and 68.63% for T5). In conclusion, putrescine solutions inoculated in ovo has a negative impact on hatchability rate, yet does not significantly influence REWxRCW.

Key Words: Broiler breeder, broiler chicken, hatchery, nutrition in ovo

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201 Effects of broiler breeder maternal age on gene and protein expression in the liver and abdominal fat of the hen and the liver and yolk sac membrane of the embryo. Antonio Beitia*, Nirun Boonsinchai, Justina V. Caldas, Katie Hilton, Pramir Mahajan, Jordan Weil, Nawin Suetsuajit, Diego Martinez, Cole Umberston, Craig N. Coon, *Center of Excellence for Poultry Science, Curitiba, Paraná, Brazil.
Breeder hen age affects yolk size, hatching weights, as well as the growth rate of progeny. Yolk sac membrane (YSM) and liver (LV) are two of the main tissues functioning for nutrient utilization during embryonic development. The objective of this study was to understand the differences in nutrient utilization between embryos from young and old breeder hens. An RT-qPCR was performed on experiments (Exp) 1 and 2. Data were analyzed using the delta-delta Ct method. Western blot analyses were performed to quantify the expression of different proteins. In Exp 1, YSM and LV were taken from embryos on an embryonic day 17 (E17) received from hens at 26wk (young or treatment) and 35wk (old or control) of age and were analyzed on triplicate samples. In Exp 2, LV and abdominal fat were taken from hens of each age and were analyzed on triplicate samples. A total of 48 selected genes relating to fatty acid, glucose, and amino acid metabolism were profiled from each sample. The fold regulation comparison was performed between treatment and control groups with fold regulation cut off at 2.0 and p-value cut off at 0.05. In Exp 1 the over-expressed in YSM of the embryo from young hens were PC, GPT2, LPL with fold regulation of 75.45, 28.00, and 23.93, respectively (p<0.05). Genes that were under-expressed in YSM, but overexpressed in the embryonic liver were FABP2, LRP2, and PKM2 with fold regulation of 1054.61, 191.67, and 9.27, respectively (p<0.05). In Exp 2, the dominant over-expressed genes in the liver of young hens over the control were fatty acid metabolism genes SCD, ELOVL6, and ACACA with fold regulation of 7.27, 4.50, and 4.41, respectively (p<0.05), while there were only a few genes that under-expressed: ACAD11, PCK1, and H6PD (p<0.05). The over-expressed genes in abdominal fat were LPL, G6PC2, and CD36 (p<0.05), whereas the under-expressed genes were ACAD11, FBP1, and PC (p<0.05). Older hens had a higher protein abundance of FABP on the YSM, but a lower abundance of LRP2, GPT2, PC, and LPL (p<0.005). Younger hens showed a higher protein abundance in the YSM of LRP2, GPT2, PC, and LPL (p<0.005). In conclusion, the study shows the age effects on the expression of genes related to fatty acid and carbohydrate metabolism. Furthermore, these findings indicate that the E17 from younger hens is unable to properly mobilizing fatty acids in comparison to the E17 from older hens, but instead utilizes particular genes related to carbohydrate metabolisms such as PC and GPT2 to mobilize specific nutrients for glycolysis during embryonic development.

Key Words: Broiler breeder, fatty acids, carbohydrates, gene expression, Protein expression

202 Feeding different cultivars and quality levels of faba bean to broiler chickens. Miranda N. Smit*, Liangfei He2, Eduardo Beltranena1,2, Livestock and Crops Research Division, Alberta Agriculture and Forestry, Edmonton, Alberta, Canada, Agricultural, Food & Nutritional Sciences, University of Alberta, Edmonton, Alberta, Canada.

A concern of both pulse growers and poultry producers is how frost damage around harvest time can affect the feeding quality of faba bean. To investigate, two cultivars of zero-tannin (Snowbird, Snowdrop) and one of low vicine/convicine (Fabelle) faba bean were sourced from certified seed growers and planted at a single site (53°38'52.2" N 113°21'09.2" W) late May and desiccated early October to purposely increase the proportion of damaged beans at harvest. The 3 sourced certified parent seed cultivars, the 3 harvested frost-damaged bean cultivars and a control diet were then fed to 740 broiler chickens housed in 63 floor level pens in a 2 x 3 factorial design plus control (9 pens of 11-12 birds per treatment). Starter (d 0-11), grower (d 12-24) and finisher (d 25-40) phase diets included 15, 30 and 45% faba bean in partial (starter, grower) or total (finisher) replacement of soybean meal (SBM; control diet) and wheat grain. There was a cultivar x bean quality interaction (P < 0.05) on daily feed disappearance (ADFI) and feed efficiency (FE). Broilers fed low-quality Snowdrop consumed 1.0 g/d more Finisher and 6 g/d more feed overall than those fed low-quality Snowbird or Fabelle; broilers fed certified parent seed were intermediate. However, daily weight gain (ADG) and bird body weight (BW) at the end of each growth phase were not affected by either cultivar or bean quality. Controls fed SBM only grew 2.75 g/d faster overall and were 113.5 g heavier at the end of the trial than broilers fed faba bean (P < 0.05). Feeding low-quality Fabelle resulted in best overall trial FE (0.646) vs. certified parent seed Snowbird (0.611), Fabelle (0.624) or low quality Snowdrop (0.624). Controls fed SBM only had 0.024 g/g greater overall FE than broilers fed faba bean (P < 0.05). Feeding certified parent seed or frost-damaged beans had no effect on antemortem live BW, chilled carcass wt, dressing percentage or yield of saleable cuts as proportion of chilled carcass wt except that broilers fed Snowbird or Snowdrop had 0.8% unit larger thighs than those fed Fabelle (P < 0.05). Controls fed SBM only were 110 g heavier at slaughter, had 72 g heavier chilled carcass wt and 0.5% unit greater dressing percentage than broilers fed faba bean (P < 0.05). The results of this experiment indicate that feeding Frost-damaged faba bean, to the extent observed in this trial, did not adversely affect growth performance or carcass attributes of broiler chickens compared to feeding the certified parent seed bean quality of these faba bean cultivars.

Key Words: Antinutritional factors, Broiler chicken, Carcass cuts, Faba bean cultivars, Growth Performance

203 Dietary supplemental full-fatted and defatted microalgae Desmodesmus sp. exerted similar impacts on growth performance and gut health of broiler chicks. Tao Sun1,2, Benjamin Wyman1, Guanchen Liu1, Colin Beal1, Schonna Manning2, Zackary I. Johnson2, Xin Gen Lei1, Animal Science, Cornell University, Ithaca, New York, USA.
The marine microalgae *Desmodesmus* sp. have shown promise as a feedstock for biofuel production. Towards characterizing the broader revenue potential, we performed two experiments to determine the impacts of supplementing the full-fatted (C046, 41% crude protein) or defatted (LEA, 39% crude protein) biomass of *Desmodesmus* sp. on growth performance and gut health of broiler chicks. A total of 216 and 180 Cornish male chicks (day-old) were divided into three dietary treatment groups (12 cages/treatment) in Experiments 1 (6 chicks/cage) and 2 (5 chicks/cage), respectively. The three experimental diets were: 1) a corn-soybean meal basal diet (BD), 2) BD + 5% C046, and 3) BD + 5% LEA. All chicks were housed in environmentally-controlled rooms and given free access to water and respective diets for two weeks. Growth performance was measured weekly in both experiments. Blood, liver and duodenum samples were collected (2 chicks/cage) at the end of Experiment 2 after chicks were orally gavaged with two consequentially daily doses of 2 mL of dextran sulfate sodium (DSS, 0.5 g/mL water) or 0.3 mL of lipopolysaccharide (LPS, 0.5 mg/mL water) to induce leaky gut in chicks (phosphate-buffered saline as administration control). Data were analyzed using one-way ANOVA and Tukey multiple mean comparisons. In Experiment 1, supplementing C046 and LEA enhanced (P<0.05) average daily weight gain (ADG) by 21.6% and 12.7%, respectively, compared with the control diet. In Experiment 2, the C046 diet elevated (P<0.01) ADG by 30%, and the C046 and LEA diets improved (P<0.01) feed use efficiency by 15% and 11%, respectively, compared with the control diet. Relative mRNA abundances of interleukins (IL)-1, 6 and 8 in the duodenum were 55% to 2.2-fold greater (P<0.01) in chicks fed the C046 diet than those fed the control diet. The mRNA level of tumor necrosis factor α (TNF-α) was 52% higher (P<0.05) in the duodenum of chicks fed the C046 diet than those fed the control diet. In contrast, hepatic mRNA levels of IL-1, 8 and TNF-α were decreased (6 to 71%, P<0.05) by the C046 and LEA diets compared with the control diet. The amount of claudin-1, a biomarker of gut integrity, in the duodenum was elevated (50%, P<0.05) by the C046 and LEA diets than the control diet. The amount of nuclear factor, erythroid 2 like-2 in the duodenum was elevated (43%, P<0.05) by the LEA diet than the control diet. In conclusion, the moderate supplementation of the full-fatted (C046) and defatted (LEA) *Desmodesmus* sp. biomass into a corn-soybean meal basal diet produced similar improvements of growth performance and intestinal integrity, despite elevated inflammatory responses to the administrations of DSS and LPS (Supported by DOE#DE-EE0007091).

**Key Words:** Broiler chicks, Growth performance, Gut integrity, Inflammation, Microalgae

### 204 Effect of the inclusion of enzyme-treated soy protein in starter diets of two different amino acid density feeding programs on performance of broiler chickens

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The objective of this study was to evaluate the performance effect of the addition of an enzyme-treated soy protein (ESP, Hamlet Protein A/S, Denmark), with a very low anti-nutritional factor content, in broiler starter diets (d0-14) in the frame of two different amino acid (AA) density feeding programs (100% and 88% of the 2019 Cobb 500 recommendations) at three commercially significant market ages (d35, 42, and 49). A total of 1104 day-of-hatch Cobb 500 male broiler chickens were used to assess the effect of ESP inclusion in starter feed and AA density on growth performance. Dietary treatments consisted of a 2x2 factorial arrangement with 2 protein sources (SBM or SBM+ESP) x 2 AA densities (100% and 88%), resulting in four starter diets (12 replicates/diet; 23 chicks/replicate): T1 (33.36% SBM, 100% AA density); T2 (27.65% SBM, 5% ESP, 100% AA density); T3 (27.17% SBM, 88% AA density) and T4 (21.23% SBM, 5% ESP, 88% AA density). In the grower (d14-35) and finisher (d35-49) periods, each respective AA density group was fed the same corn-SBM based diet (T1 and T2: 100% AA density; T3 and T4: 88% AA density). Feed and water were provided ad libitum. Body weight and feed intake by pen were recorded at d0, 14, 28, 35, 42 and 49. Weight gain and feed conversion ratio (FCR) were calculated. FCR was adjusted for mortality and fixed BW (d35=2.25kg, d42=2.94kg, d49=3.40kg). Data were analyzed using the statistical software R (R Core Team 2020 version 4.0.0) by 2-way ANOVA. Differences were considered significant at p<0.05. No interaction between ESP inclusion in starter feed and AA density level was observed for weight gain or mortality- and BW adjusted FCR on days 35, 42 and 49. However, ESP inclusion in starter feed had a significant effect on day 49 on mortality adjusted (SBM=1.73, SBM+ESP=1.71; p=0.03) and BW adjusted FCR (SBM=1.75, SBM+ESP=1.70; p=0.04). AA density levels significantly affected weight gain at day 35 (p=0.05). At day 35, 42 and 49, mortality- and BW adjusted FCR were significantly improved by increased AA density level (p=0.05). In conclusion, replacing part of the SBM in starter diets for broiler chickens with 5% ESP affected performance on day 49 by improving mortality- and BW adjusted FCR by 2 and 5 points. The 100% AA density recommendation improved FCR significantly at all three commercial market ages when using SBM only and SBM + ESP diets. No interaction was observed between ESP inclusion in starter diets and AA density level on performance of broiler chickens at the three commercially significant market ages (d35, 42 and 49).
205 Effects of cellulose and soybean hulls as sources of dietary fiber on the growth performance, nutrient digestibility, gut morphology, and intestinal gene expression of broiler chickens. Oscar J. Tejeda, Woo Kim, Poultry Science, The University of Georgia, Athens, Georgia, United States.

Dietary crude fiber (CF) is an intrinsic component in plant feedstuffs that have been associated with physiological and functional changes in the gastrointestinal tract. Therefore, the objective of this study was to evaluate the effect of dietary fibers in the form of purified cellulose (solka floc® SF) or soybean-hulls (SH) on growth performance, nutrient digestibility, gut morphology, and intestinal transporter gene expression of broiler chickens. A total of 420 one-day old Cobb® male broilers were randomly assigned to seven dietary treatments and reared to 20 days of age in battery cages (n=6 replicates per treatment). The control group consisted of a simple corn and soybean-meal based diet. The six fiber treatments had increasing amounts of SF or SH to achieve 4, 6, and 8% crude fiber. Chromium oxide was added as an indigestible marker at 0.3% in all treatment diets from 14 to 20 d for nutrient digestibility analyses. Intestinal samples from the duodenum, jejunum, and ileum were taken on d20 for morphology analyses. Growth performance was measured weekly. At 20 d, six birds per cage were euthanized, and ileal digesta samples were collected from five birds for nutrient digestibility analyses and ileal samples from one bird for gene expression. Data were analyzed by one-way ANOVA using JMP® 2019. Birds fed the 4% SH diet had a higher d20 body weight gain compared to those fed 8% CF regardless of fiber sources (P=0.0118). Control and 4% SH groups had the best feed conversion ratio among treatments at 7, 14, and 20 d (P<0.05). Birds fed 8% SH diets had the highest duodenal villi height among all treatments (P<0.001). Birds fed the control and 4% SH had the highest jejunal villi height among treatments (P<0.001). Birds fed the 4% SF and 4% SH had the highest ileal villi height among treatments (P<0.001). No statistical differences in GLUT2 (glucose transporter), SLC7A9 and SLC7AL (amino acid transporters) were observed among treatments (P>0.05). No differences in energy and crude protein digestibility were observed among treatments (P>0.05). Dry matter digestibility was higher in 6% SF compared to 8% SH (P=0.0105). Birds fed 8% SH had the highest methionine digestibility among treatments (P<0.001). Birds fed 6 and 8% SH had the highest threonine digestibility among treatments (P<0.001). In conclusion, fiber type is a crucial factor regulating intestinal development, nutrient digestion, and growth performance.

Key Words: Enzyme-treated soy protein, Soybean meal, Amino acid density, Broiler chickens, Performance


A proprietary fermentation process of soybean meal (SBM) was developed at a Central America oilseed processing plant. In brief, the process is based on a dual solid-state fermentation of Hi-Pro SBM using a combination of specific Aspergillus spp. and Bacillus spp. inoculum. Fermentation took place under semi-anaerobic conditions and prevailing ambient temperatures (25 – 35°C). Moisture levels during fermentation were maintained at 55% and fermentation progress was controlled through repeated measures of temperature, pH and sugar content. Fermentation was halted by low temperature drying to humidity levels of 10%. The objective of this presentation is to report the effect of fermentation on the net content of heat-labile and heat-stable antinutritional factors of soybean meal. Student's t-test was used to differentiate the analytical means of SBM versus those of FSBM. The results for at least six industrial productions of fermented soybean meal versus their unfermented SBM were as follows: trypsin inhibitors significantly decreased (P<0.001) from 2.56±0.42 mg/g in SBM (N=8) to 0.97±0.14 mg/g in FSBM (N=6). Sucrose significantly decreased (P<0.001) from 5.91±0.87% in SBM (N=6) to 0.5±0.04% in FSBM (N=26). Raffinose significantly decreased (P<0.001) from 1.28±0.45% in SBM (N=6) to 0.08±0.07% in FSBM (N=26). Stachyose significantly decreased (P<0.001) from 4.86±0.94% (N=6) to 0.13±0.09% in FSBM (N=26). Fermentation also resulted in an important reduction in lectin content and increase in organic acid content most notably lactic acid from non-detectable in SBM to 6.16±0.22% in FSBM (N=13). Similarly, fermentation also increase acetic acid from non-detectable in SBM to 0.52±0.27% in FSBM (N=13). In conclusion, fermentation of SBM – by the process as described above – improves the nutritional characteristics and should allow for greater incorporation of diets in young and sensitive.

Key Words: fermented soybean meal, trypsin inhibitors, stachyose, raffinose, lactic acid

207 Effect of early posthatch supplementation of vitamin E and omega-3 fatty acids on the severity of wooden breast, breast muscle morphological structure, and gene expression in the broiler breast muscle. Ji Wang*, Daniel Clark, Sheila Jacobi, Sandra Velllemam, The Ohio State University, Wooster, Ohio, United States.

The wooden breast myopathy (WB) has arisen primarily in pectoralis major (p. major) muscle of commercial broilers and results in significant economic losses. It is characterized by palpation of a rigid p. major muscle that is under severe oxidative stress and inflammation. Previous studies have
shown that vitamin E (VE) has antioxidant properties and omega-3 (n-3) fatty acids have an anti-inflammatory effect. Our preliminary data showed that supplementation of VE early posthatch reduced the severity of WB. The objective of this study was to further examine the effects of VE and n-3 fatty acids on breast muscle morphological structure and expression of genes associated with WB. A total of 210 Ross 708 broilers were divided into 7 treatments with 10 replicates of 3 birds each. The control group was fed a standard diet during the entire study (0 to 58 D). Supplementation of VE (200 IU/kg), n-3 fatty acids (n-6/n-3 ratio of 3:1), or combination of both was performed during the starter phase (0 to 10 D) or grower phase (11 to 24 D). All of the broilers were harvested at 58 D. The p. major muscle samples were collected, processed, cross sectioned, stained, and images taken. Muscle fiber width was measured as the diameter (µm) of myofibers. Perimysial and endomysial connective tissue space were determined as the width (µm) of the perimysium and endomysium, respectively. Morphology score was obtained with higher scores representing more well-structured myofibers. Microscopic WB score was obtained based on degree of necrosis and fibrosis. Morphological attributes were analyzed with PROC Mixed and scores were analyzed with PROC Glimmix procedure of SAS. Expression of 21 genes associated with WB was determined with Nanostring analysis. More giant myofibers (fiber width ≥ 70 µm) were found in the group supplemented with VE and n-3 fatty acids in the starter diet compared to supplemental VE group in the grower diet (P = 0.02). Vitamin E supplementation in grower diet increased morphology score compared to n-3 fatty acids supplementation in the grower diet (P = 0.04). Broilers fed VE in the grower diet had a 15% increase of muscle with no WB compared to the control group (P = 0.05). Genes related with muscle growth and response to inflammation were differentially expressed in the group supplemented with VE in the grower diet compared to the control group (P ≤ 0.05). For example, expression of selectin E was increased when broilers were fed VE in the grower diet (P = 0.02). Taken together, muscle morphological results were consistent with changes in gene expression indicating VE supplementation during the grower phase could reduce incidence of WB by up to 15% which would be of great benefit to the broiler industry.

Key Words: wooden breast, muscle morphology, omega-3 fatty acid, vitamin E, broiler

208 The effects of maternal fish oil supplementation on offspring-broiler growth performance, body composition and bone microstructure at market age. Yuguo H. Tompkins1, Chongxiao Chen1, Jeanna Wilson1, Brynn H. Voy2, Woo Kim1,1 Poultry Science, University of Georgia, Athens, Georgia, United States, 2Department of Animal Science, University of Tennessee, Knoxville, Tennessee, United States.

Maternal supplementation of fish oil, which contains high levels of long-chain omega-3 polyunsaturated fatty acids, has been shown to benefit the growth and bone formation of offspring in humans and mice, however, the effect in poultry still remains unknown. In order to evaluate the effect of maternal dietary fish oil on growth performance, body composition, and bone quality in market-age broilers, breeder hens were fed the experimental diets containing 2.3% food-grade soybean oil (SO) or fish oil (FO) for 4 wks. Fertilized eggs were collected over a period of 7 days and incubated under standard conditions. A total of 240 one-day-old chicks from different maternal diet groups (SO group and FO group) were randomly allocated in 12 floor pens (6 replicates/ 20 birds). Growth performance and the feed conversion ratio (FCR) were recorded and calculated at d1, 14, 28 and 42. On d 42, 3 birds per pen were randomly selected for body composition measurement by a Dual Energy X-ray Densitometry (DEXA), and the femurs were collected for bone microarchitectural analyses by Micro-CT. Femoral bone marrow was collected to measure the expression of key osteogenic and adipogenic genes by qPCR, and means were compared by student’s t-test (P<0.05). Maternal FO supplementation significantly improved the body weight and body weight gain in offspring broilers at the finisher stage (P<0.05) and overall period (P<0.05) but didn't affect FCR. A higher percentage of body fat (P<0.05) and a higher percentage of lean mass (P<0.05) were observed in the FO group by DEXA at d 1 and d 42, respectively. Bone microstructural analysis indicated that there were no significant differences in total bone mineral content (BMC), bone mineral density (BMD), or trabecular microstructure of the distal end of the femurs. However, in the middle shaft of femurs, the FO group had significantly lower cortical porosity (P<0.05), smaller pore space volume (P<0.05), lower total porosity percentage (P<0.05), lower open porosity percentage (P<0.05), and smaller open-pore space volume (P<0.05), but a higher number of closed-pore (P<0.05) and a trend of higher cortical BMD (P= 0.054). Moreover, maternal FO supplementation suppressed the expression of adipogenesis-related genes such as PPARγ (P<0.05), FABP4 (P<0.05), and C/EBPβ (P<0.05), but did not significantly affect osteogenesis gene expression in the bone marrow. Based on those findings, the maternal fish oil intake not only enhanced body composition and lean mass in offspring, but also decreased adipogenesis in the bone marrow that coupled with lower porosity on the cortical bone which indicated an improvement in bone quality.

Key Words: long chain omega-3 polyunsaturated fatty acids, Maternal fish oil, body composition, broilers, bone health

209 Growth performance and carcass characteristics of MV × Cobb 700 broilers fed diets varying in apparent metabolizable energy from 34 to 46 days of age. Stephanie Philpot1, Justina V. Caldas2, William A. Dozier, III1, Poultry Science, Auburn University, Auburn, Alabama, United States, 2Cobb Vantriss, Siloam Springs, Arkansas, United States.
Limited data are available defining the optimum apparent metabolizable energy (AMEₙ) specification of MV × Cobb 700 broilers during the finisher phase. An experiment was conducted to determine the effects of AMEₙ on growth performance, carcass characteristics, and breast quality of MV × Cobb 700 broilers from 34 to 46 D of age. Fourteen hundred and eight broilers were vent sexed and randomly distributed by sex into 64 floor pens (22 birds; 0.10 m²/bird). A 2 × 4 factorial arrangement of treatments was used, with male and female broilers fed diets formulated to AMEₙ of 3,108, 3,152, 3,196, or 3,241 kcal/kg from 34 to 46 D of age. Each treatment was represented by eight replicate pens. Broilers were fed common starter and grower diets (1 to 18 and 19 to 33 D of age, respectively) formulated to the primary breeder guidelines. Corn, soybean meal, and dried distillers grains with solubles were the primary ingredients during all feeding phases. Body weight gain, feed intake, feed conversion ratio, and mortality were determined from 34 and 46 D of age. At 47 D of age, 12 birds per pen were processed and the weights and yields of the abdominal fat, carcass, and breast fillets and tenders were assessed. Breast fillets were evaluated for prevalence and severity of wooden breast and white striping using a subjective scoring system of normal, mild, moderate, or severe defects. Data were analyzed with a two-way analysis of variance. No interactive effects (P ≥ 0.07) were observed between sex and AMEₙ for growth performance or processing characteristics. A sex × AMEₙ interaction was observed for white striping where males fed diets containing 3,152 kcal/kg had increased (P = 0.038) incidence of moderate white striping compared with females fed diets containing 3,108 kcal/kg. For the main effect of AMEₙ, broilers fed diets containing 3,196 kcal/kg had higher BW gain (P = 0.032), reduced feed conversion ratio (P = 0.020), and reduced abdominal fat weight and percentage (P ≤ 0.045) compared with birds fed diets containing 3,108 kcal/kg. Breast meat yield was highest in broilers fed diets containing 3,108 (P = 0.0030) compared with broilers provided with all other treatments. Breast quality characteristics were not affected by AMEₙ (P ≥ 0.11). Males had higher BW gain and feed intake, lower feed conversion ratio, heavier carcass, breast fillets, and tenders, and increased incidence of wooden breast scores of moderate and severe compared with females (P ≤ 0.001). These results indicate that increasing AMEₙ above 3,196 kcal/kg in MV × Cobb 700 broilers has little impact in performance or breast quality characteristics during the finisher period, which may allow for reductions in feed cost.

**Key Words:** apparent metabolizable energy, body weight gain, breast quality, broiler, feed conversion ratio

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The present study aimed to evaluate the effects of putrescine-based nutritional solutions on weight of organs and intestinal morphology at hatching, as well as on growth performance of broiler chickens in the initial phase. Four nutrient solutions with different concentrations of putrescine (T2: 0.015%; T3: 0.030%; T4: 0.060% and T5: 0.090%) and a control group without inoculation (T1) were evaluated. A total of 640 eggs from Ross® commercial lineage were selected (~64.42g) and incubated according to its guidelines. Each treatment included 128 eggs randomly distributed in the incubator. On the 17th day of incubation, the eggs were injected with 0.5 ml of the putrescine solution in the amniotic fluid of the embryo, according to each treatment. After 504 hours of incubation, 400 chicks were housed in a randomized block design, with 5 treatments and 8 replicates of 10 birds each. Water and feed were provided ad libitum, and diets were identical for all treatments. At birth, 6 chicks from each treatment were euthanized to determine the weight of the yolk sac, breast, liver, and intestine, and its percentage to the animal’s body weight. The height of the villus (VH) and depth of the crypt (CD) in the chicks’ jejunum were also measured. Average feed intake (AFI), average weight gain (AWG), and feed conversion (FC) were determined at 7 days of age. To determine the best levels of putrescine, the polynomial regression model and ANOVA were used. The weight of yolk sac, breast, liver, and intestine at birth, did not show statistically significant differences between the treatments (P > 0.05). In the morphological analysis of the jejunum, VH did not show statistical difference (P > 0.05), while CD (µm) showed a significant difference (P = 0.0001). For the increasing levels of putrescine, the quadratic effect was observed on CD (T1: 57.63, T2: 59.26, T3: 62.28, T4: 79.06, and T5: 78.80). Average feed intake (AFI), AWG, and FC at 7 days of age did not differ (P > 0.05). Therefore, it is concluded that putrescine-based solutions inoculated in ovo did influence the depth of the crypt in the jejunum of chicks. However, it did not significantly influence the organs’ weight at birth and growth performance of broiler chickens at 7 days old.

**Key Words:** Putrescin, incubation, nutrition, growth, broiler

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### 211 Maternal fish oil alters adipose development in the broiler chick embryo.

Minjeong Kim*, Usuk Jung, Kamille Piacquadio, Suchita Das, Liesel Schneider, Jeanna Wilson, Brynn H. Voy, 1Department of Animal Science, The University of Tennessee, Knoxville, Tennessee, United States. 2Department of Poultry Science, University of Georgia, Athens, Georgia, United States.

Hen diets can potentially be used to improve body composition in broilers through developmental programming. We previously demonstrated that enriching the broiler-breeder hen diet in fish oil, a rich source of long chain omega-3 polyunsaturated fatty acids (n-3 PUFA; eicosapentaenoic acid, EPA; 20:5 n-3 and docosahexaenoic acid, DHA; 22:6 n-3) reduced fat accretion and adipocyte...
size in chicks at 14 days of age compared to a corn oil-based hen diet. Expression of genes that regulate adipogenesis were also downregulated, suggesting that EPA and DHA interact with the mechanisms that control adipose development. Here we tested the hypothesis that enriching the hen diet in fish oil specifically alters adipose development in the embryo, without compromising embryo growth or muscle development. Broiler-breeder (Cobb 500) hens were fed diets containing 3% fat from fish oil (FO), as a source of EPA and DHA, or soybean oil (SO) as an n-6 PUFA control for four weeks. Fertilized eggs were incubated to embryonic ages E12, E14, E16, E18, and E20. At each age, eggs were weighed and embryos (n=10/diet) were euthanized and weighed, along with yolk sac. Fat pads (parafemoral subcutaneous), and breast muscle (pectoralis major) were dissected and weighed, then fixed in formalin or snap-frozen for subsequent histological and molecular analyses, respectively. At E20, samples of adipose tissue were also used for whole mount measurement of adipocyte size and number. Tissues were imaged with an EVOS XL Core Imaging System and analyzed using ImageJ (V 1.52a; NIH.gov). Completely randomized design with factorial treatments of diet and development age was implemented. Mixed model analysis of variance (PROC GLIMMIX SAS 9.4) was used to test fixed effects of dietary treatment, age, and their interaction, as well as adjust least squares means for the covariate of set egg weight. Set egg weight was removed if not significantly related to the response of interest. Then, the means were compared by Tukey’s significant different test (p < 0.05). Weights of egg, yolk sac, embryo, adipose, and breast did not differ significantly between SO and FO at any time point. At E20, however, MFO significantly reduced adipocyte size, increasing the frequency of small adipocytes (p < 0.05). These results support the hypothesis that EPA and DHA in the hen diet specifically alter adipose development in the chick embryo. Efforts to identify the molecular mechanisms that underlie developmental programming of fat accretion in broiler chicks are underway. In conclusion, in combination with our previous work, these data suggest that developmental programming through the hen diet is a valuable and efficient tool with which to improve broiler performance.

Key Words: Broiler, Body Composition, Embryo, Fish Oil, Maternal Diet

212 Evaluation of Tasco (Ascophyllum nodosum) on the growth performance of mixed-sex broiler chickens. Robin Hilchie, Bruce Rathgeber, Stephanie Collins, Joshua Gong, Janice MacIsaac, Animal Science and Aquaculture, Dalhousie University, Truro, Nova Scotia, Canada, Agriculture and Agri-Food Canada, Guelph, Ontario, Canada.

Replacements for antibiotics which produce comparable growth need to be evaluated and made available to poultry producers. Tasco a sun-dried brown seaweed (Ascophyllum nodosum) is a potential prebiotic. The objective of this study was to determine the effect of Tasco on the growth performance of broiler chickens. The study was designed as a one-way analysis with dietary supplement as the main effect (no supplement, commercial antibiotic, 0.25, 0.5, 0.75 and 1% Tasco). Two replicate trials were conducted. A total of 1248 d-old mixed sex chicks for each trial were placed in 48 floor pens and grown until 34 d of age. Feed consumption, weight gain and feed conversion efficiency were measured from 0-14, 15-25 (23 Trial 2) and 26 (24 Trial 2)-34 d of age. Birds were weighed on days 14, 25 (23) and 34. Data was analysed by Proc Mixed procedure of SAS. There was no effect (P>0.05) of supplement on feed consumption for Trial 1. Birds fed 0.25, 0.5 and 1% Tasco had heavier (P<0.05) 14-d weights; birds fed 0.25 and 1% Tasco had heavier (P>0.05) 25-d weights than those fed antibiotic, no supplement and 0.75% Tasco; birds fed 1% (2259 g) and 0.25% (2241 g) Tasco had heavier 34-d weights than those fed no supplement (2165 g) and the antibiotic (2154 g). Birds fed 0.5% Tasco were more efficient (P<0.05) (1.36 feed gain1 bird) than those fed the 0.75% Tasco (1.48 feed gain1 bird) during the 1-14-d period. Feed efficiencies were similar (P>0.05) among the treatments during the 15-25 and 26-34-d periods. For Trial 2 there were no effects (P>0.05) of supplement on 1-14 and 15-23-d feed intakes or 14 and 23-d body weights. Birds fed the 1% Tasco consumed more (P<0.05) feed (1846 g bird-1) than those fed the antibiotic (1757 g bird-1), no supplement (1737 g bird-1), 0.5% Tasco (1763 g bird-1) and 0.25% Tasco (1754 g bird-1) treatments during the 24-34-d period. Birds fed 0.75% Tasco (2295 g bird-1) had heavier (P<0.05) 34-d weights than those fed the nonmedicated (2163 g), antibiotic (2190 g), 0.25% Tasco (2149 g) and 0.5% Tasco (2160 g) treatments. Body weights were similar (P>0.05) for the birds fed 0.75% and 1% Tasco (2281 g). Birds fed 1% Tasco and the antibiotic had similar (P>0.05) weights. Birds fed 1% Tasco were more efficient (P<0.05) (1.32 feed gain1 bird) than those fed the 0.25% Tasco (1.41 feed gain1 bird) for the 1-14-d period. Feed efficiencies were similar (P>0.05) among the treatments during the 15-23 and 24-34-d periods. Results from this study indicated that the inclusion of Tasco in the diet can improve growth performance compared to unsupplemented and antibiotic controls.

Key Words: Broiler, Growth performance, Seaweed, antibiotic

213 Determination of daily dietary energy utilization for maximal protein deposition in fast-growing male broilers from 3 to 18 days of age. Nawin Suesuttajit, Antonio Beitia, Jordan Weil, Pramir Maharjan, Diego Martinez, Craig N. Coon, Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States.

Modern broilers today have the potential to gain body protein quickly through nutrient intake during starter and grower periods. However, protein deposition requires adequate calorie intake in combination with digestible amino acids for maximum accretion. Research is needed to
understand when and how much additional energy intake is needed for maximum protein deposition in order to better design feeding programs for feeding phases. Two studies were conducted to determine the daily dietary energy utilization for protein deposition by using dual energy x-ray absorptiometry (DEXA) (GE Lunar Prodigy). In Exp1, a total of 1440 fast-growing male broilers were randomly assigned to 48 floor pens and fed one of two test diets from d3 to d10 throughout an 8-day experimental period (8 trts; 6 reps). Each treatment consisted of feeding one diet (Diet 1) for a set period of days and then switching the diet to a higher energy diet (Diet 2). Diet 1 was formulated to Cobb500 starter recommendations (ME 3086 Kcal/kg and 3.40 g dLys/Mcal) while the Diet 2 contained 50 extra calks ME/kg and a constant protein to energy ratio, similar to the ratio in Diet 1. At d10, broilers were scanned to determine the dietary energy intake needed for maximum protein deposition. In Exp2, a total of 1440 fast-growing male broilers were randomly assigned to 48 floor pens and fed one of two test diets from d9 to d16 throughout an 8-day experimental period (8 trts; 6 reps). Each treatment consisted of feeding one diet (Diet 1) for a set period of days and then switching the diet to a higher energy diet (Diet 2). Diet 1 was formulated to Cobb500 grower recommendations (ME 3086 Kcal/kg and 3.40 g dLys/Mcal) while the Diet 2 contained 50 extra calks ME/kg and a constant protein to energy ratio, similar to the ratio in Diet 1. At d17, broilers were scanned to determine the dietary energy intake needed for maximum protein deposition. Data was analyzed one-way ANOVA using JMP Pro 14 (SAS Institute, 2018). Exp1 showed body weight, lean mass, protein mass significantly increased when the diet was changed to a higher energy containing diet on d6 (P<0.05). The results in Exp2 presented that the protein mass significantly increased in the treatment that had diet changes on d16 (P<0.05). These studies indicate that d6 was the age of broiler required extra energy stimulation with the same amino acid ratio to energy from hatch through d16 and the next broiler age that needed energy stimulation response was d16 with diet stayed the same amino acid ratio to energy. This model would develop accurate phase feeding of broiler diets throughout 42 days in further.

Key Words: Broilers, DEXA, Energy utilization, Calories, Protein deposition

214 Effects of supplementation of almond hulls on growth performance, lesion score and anti-oxidative enzyme activity in Eimeria-challenged broilers. Jinquan Wang*, Fanbin Kong1, Woo Kim, 1Poultry Science Department, University of Georgia, Athens, Georgia, United States, 2Food Science and Technology, University of Georgia, Athens, Georgia, United States.

Almond hulls are by-products from almond industry with a high amount of fermentable sugars, dietary fibers, and antioxidants. An experiment was conducted to evaluate diet formulating with almond hulls on growth performance, lesion score and superoxide dismutase (SOD) activity in Eimeria-challenged broilers. A total of 360 one-day old Cobb500 male broilers were randomly allocated into 5 dietary treatments with 6 replicates and 12 birds each. Five dietary treatments consisted of T1= a non-challenged control that birds fed a corn and soybean meal diet; T2= Eimeria-challenged birds fed the same diet as the non-challenged control; or T3-T5= diets formulated to contain 3, 6, and 9% of almond hulls, respectively, fed to Eimeria-challenged birds. All the dietary treatments were formulated to meet or exceed Cobb nutrient requirements. Broilers in challenged group (T2 to T5) were inoculated with 25,000 oocysts of E. maxima, E. tenella and 125,000 oocysts of E. acervulina on d14, and birds in non-challenged group were inoculated with the same amount of PBS. At d20, 5 birds from each replicate were sacrificed for lesion score. Liver sample was collected from 1 bird per replicate for SOD enzyme activity. Body weight and feed intake were recorded at d14, 20, and 26 for calculating body weight gain and feed conversion ratio. The study was conducted as a completely randomized design and all data were analyzed using one-way ANOVA model by SAS. There is no difference in body weight gain, feed intake, and feed conversion ratio during d0-14 and d21-26 of age between treatments. Birds were challenged with Eimeria spp. (T2 to T5) showed a worse (P<0.01) body weight gain, feed intake, and feed conversion ratio compared to non-challenged control for 6 days post-challenged period. The diet containing 9% almond hulls (T5) showed a higher (P<0.05) cecal lesion score compared to challenged control (T2) at d20. Eimeria-challenged control (T2) decreased liver SOD enzyme activity at d20 compared to non-challenged control group (T1), whereas diets containing 3 and 9% almond hull improved (P<0.05) liver SOD enzyme activity compared to challenged control. All the diets containing almond hulls reached to a similar level in SOD enzyme activity compared to the non-challenged birds. In conclusion, diets containing almond hulls improved liver SOD enzyme activity but had no effect on growth performance and lesion score in Eimeria-challenged birds.

Key Words: Broiler, Eimeria-challenged, Almond hull, growth performance, antioxidant

215 Application of NutriOpt® formulation strategies for economic performance enhancement in broiler chickens. Hugo Romero-Sanchez*1,2, Yanming Han1,2, Greg Page1, 1Trouw Nutrition USA, Wake Forest, North Carolina, United States, 2Trouw Nutrition, Amersfoort, Netherlands.

In a recent study we demonstrated that NutriOpt® strategies support performance and profitability. With the aim to add more evidence under US conditions a trial was conducted using a total of 2,736 Cobb 500 as hatched broilers, assigned to 6 treatments (12 replicates/treatment). Treatments included: 1) control (consultant-formulated typical US formulation), 2) shadow-formulated using NutriOpt® NIRS and raw material database, 3) as 2, using NutriOpt® rPhos and phytase dosing matrix and AA profile, 4) as 3, using...
NutriOpt® structure concept, 5) Optimized for margin per broiler using NutriOpt® broiler model (Margin), and 6) Optimized for margin per bird using NutriOpt® broiler model for breast meat yield (BMY). The experiment was conducted under semi-commercial conditions at NC State University, using corn-soy-DDGS- meat meal based diets fed in 3 phases: d0-d14, d15-d28, d28-d49. Body weights (BW) and feed intakes (FI) were assessed by pen on days 0, 14, 28, and 44. Carcass yield parameters were assessed on 2 randomly selected birds in each pen on day 49. All variables were subjected to ANOVA in a complete randomized block design and difference between means were partitioned using LSMEANS and statements of significance were based upon P ≤ 0.05. Formulating a shadow diet (Treatment 2), using the same ingredient constraints as the control diet, but with the NutriOpt® nutrient database, had no effect on performance metrics in any phase of the study (P>0.050), while significantly reducing feed cost/bird compared with control group (P≤0.05). Birds fed Trt 3 had significantly poorer market weights relative to Trt 2 (-2.4%), largely due to lower feed intakes between d28-44 (P<0.05). Inclusion of dietary structure (Trt 4) had no relative impacts on weight gains, feed intakes or feed conversion in the grower or finisher phases, compared with broilers fed Trt 3 (P>0.050), supporting the benefits of the structure concept in reducing feed costs. Model-optimized nutrient recommendations to minimize cost of production significantly reduced market weights (-3.5%; P≤0.05), although cost of production was reduced as intended (-8.1%; P≤0.05), without affecting yields or profitability (P>0.05). Broilers fed the BMY-optimized feed program had the highest total white meat yield (34%), which was 2.3% points higher than those fed the Trt 5 (P≤0.05), while the control group showed intermediate results (P>0.05). The shadow-formulated diet, Trt 2, showed consistently the highest profit per bird, whole carcass and BMY(P≤0.05). The present results suggest that NIRS and NutriOpt® formulation technologies can successfully be used to reduce formulation costs while maintaining performance and/or improving overall profitability.

Key Words: Modeling, NIRS, Economics, Broiler, NutriOpt

216 Ring test and validation of NutriOpt® NIR calibrations for ingredients and poultry feed in the USA. Hugo Romero-Sanchez1, 2, Lennart Oosterbaan2, 1Trouw Nutrition USA, Wake Forest, North Carolina, United States, 2Trouw Nutrition, Amersfoort, Netherlands.

The US poultry industry has widely adopted Near Infrared technology (NIR) for quality assessment of raw materials and final feed. However, concerns have developed when using calibrations from non-US laboratory. The primary concern is the discrepancy in results that might occur due to different methods or the inherent difference between labs. Also the difference between NIR results and wet chemistry labs, suggests the need to apply a bias to correct for this difference. Therefore, a ring test of 24 samples (ingredients and finished feeds) was conducted to compare three major US laboratories with a foreign laboratory. All samples were analyzed for moisture, crude protein (CP), fat, fiber, and ash and additionally measured by NIR machine using NutriOpt® calibrations. Zeta-scores, defined as the number of standard deviation from the mean, were used to define difference between labs, considering a value “≤ 2” as a satisfactory indicator of accuracy. “2-3” score is considered doubtful and “>3” is considered unsatisfactory. A two-way ANOVA, blocking by ingredient, was conducted to compare the Z-scores for all proximate variables. For the validation, from 400 NIR analysis on poultry feed, we selected 13 samples for ANOVA, showing discrepancy between formulated values and NIR results. Samples were re-analyzed in a second NIR machine using the same calibrations and submitted for wet chemistry for moisture, CP, fat, fiber and ash. All Z-scores were “≤ 2” for all 4 laboratories and all wet chemistry variables. The foreign lab showed lower moisture in corn (P<0.05), with interaction by ingredient (P<0.05). NIRS values also showed Z-scores “>2”, and not significant difference with wet chemistry was observed (P>0.05), demonstrating that no bias needs to be applied for NutriOpt® calibrations. One outlier was observed, although further microscopic analysis showed contamination of this sample. For CP content in poultry feed, NIR analysis and wet chemistry differed in 0.16% of CP (P>0.76), while formulated expected values were 2.25% and 2.82% CP lower than NIR (P<0.0001) and wet chemistry (P<0.0006). Lower deviations were observed for other nutrients (P>0.05). The data showed that NutriOpt® NIR is an accurate, and fast method for quality control on poultry ingredients and poultry feed.

Key Words: NIRS, proximate, quality, NutriOpt, wet chemistry

217 Yellow mealworm larvae (Tenebrio molitor) grown on deoxynivalenol contaminated wheat as a feed ingredient for poultry. Dilshaan Duhra2,*, Rex Newkirk, Karen Schwean-Lardner, Fiona Buchanan, Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

The purpose of this study was to determine if yellow mealworm larvae (YML) grown on wheat contaminated with a high concentration of deoxynivalenol (DON) would influence performance of broiler chickens. The YML were produced by placing approximately 300 adult beetles into containers of wheat that contained either <1 ppm DON (LDW) or 30 ppm DON (HDW). The larvae hatched from the eggs laid and grew to the harvestable weight of approximately 110 mg. Harvested YML were fasted for a minimum of 24 hours, rinsed with water and euthanized at 20°C. The YML were dried at 110°C for 40 minutes and ground to produce insect meal. The DON levels present in the insect meals were 0.145 and 0.126 ppm DON for YML ground on LDW and HDW respectively. Seventy-five mixed sex Ross 708 broilers were randomly placed into 15 cages (5 replications/treatment) and reared on one of three diets from day 0-35. At day 14, bird numbers were reduced to 4
birds/repetition. The diets consisted of a control containing no insect meal (CD) and two diets containing 5% YML that were grown on either LDW (LMD) or HDW (HMD). The diets were formulated to meet Ross 708 2019 performance standards and fed as a mash in two phases: starter/grower (0-21 days) and finisher (21-35 days). Titanium dioxide was included as a marker in the finisher diets to measure crude protein (CP) and dry matter (DM) digestibility. Excreta for analyses were collected on days 33 and 34. Feed intake (FI) and body weight (BW) were measured over the duration of the experiment and used to calculate the feed conversion ratio (FCR). On day 35, all birds were slaughtered and dissected to collect weights of the breasts, thighs, drums, wings, liver, spleen and bursa of Fabricius. A one-way ANOVA was used to assess the effect of diet on digestibility, performance and carcass traits. Crude protein digestibility was higher in the LMD and HMD treatments compared to CD (68.17, 68.61, 66.17 ±0.49%, respectively (P=0.0091)). Dry matter digestibility was higher in the HMD diet compared to the CD and LMD diets (76.80, 74.93, 74.88 ± 0.37%, respectively (P=0.0046)). Feed intake was lower in birds fed HMD compared to CD and LMD (2469.0, 2709.1, 2762.4 ± 45.4 g, respectively (P=0.0031)). Body weight and FCR did not differ between treatments. Weights of breasts, thighs, drums, wings, liver, spleen and bursa of Fabricius were not affected by diet. Diet did not influence growth, meat yield or organ weights of birds; CP digestibility was higher in diets containing YML. These results demonstrate that YML can be grown on DON contaminated wheat and produce a safe potential feed ingredient for use in poultry production.

**Key Words:** broiler, Tenebrio molitor, growth performance, insects, Deoxynivalenol

### 218 Growth response of dual purpose grower chickens fed diets containing graded levels of Black Soldier fly larvae meal.


The exploration of alternative protein sources in feed has become imperative to reduce dependence on conventional protein sources such as fishmeal (FM) using Black Soldier fly, Hermetia illucens Lin. (BSFLM), as substitute. Therefore, the aim of this study was to investigate the growth response of dual purpose grower chickens fed diets with varying levels of inclusion of defatted BSFLM. The experiment was conducted using 2 X 5 factorial arrangement in a randomized complete block design. 600 birds comprising of 300 FUNAAB Alpha and 300 Noiler 10-week old dual purpose birds previously raised in a deep litter pen were randomly assigned to five dietary treatments with six replicates containing ten birds each. The study lasted for 9 weeks during which the growth indices of the birds were investigated. Five isonitrogenous and isocaloric grower diets comprising of control diet (basal diet with 3% fishmeal without defatted BSFLM inclusion) and basal diets with one of the four levels inclusion of BSFLM (25%, 50%, 75% and 100%) as protein for protein replacement for FM were fed ad-libitum. The body weight and feed intake (FI) of the birds were assessed and recorded weekly, while the FCR were calculated. Data obtained were subjected to one way Analysis of variance using SAS (1999). Significant means were separated using Duncan’s multiple range test. Interactive effect (P<0.05) of Breeds and levels of inclusion of BSFLM were observed on the final weight (FW), body weight gain (BWG), FI and FCR of the birds. Birds fed diets containing 50% BSFLM and fish meal had significantly (P<0.05) higher FW, BWG and FCR compared to birds fed other diets. Birds fed diet with 100% BSFLM had significantly (P<0.05) higher BW and BWG (2698.08g/bird; 1349.00g/bird) relative to those fed control diet (2553.83 g/bird; 1200.83 g/bird). The feed efficiency of the birds reduced linearly (P<0.05) with increasing BSFLM. It can be concluded from this study that diets can be substituted with 50% BSFLM for improved growth of FUNAAB Alpha and Noiler grower chickens.

**Key Words:** Dual-purpose chicken, Insect meal, Black soldier fly, Body Weight Gain, Feed Efficiency

### 220 Effect of dietary supplementation with Corn Distillers Dried Grains with Solubles on production and egg characteristics of laying Japanese Quails.

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The present study aimed to evaluate the effect of dietary Corn Distillers Dried Grains with Solubles (DDGS) on laying Japanese quail in terms of egg quality and performance. A total of 240 123 days-old quails were randomly distributed to five treatment groups, with six replicates per group and eight birds per replicate. The experimental groups fed diets supplemented with 0, 6, 12, 18 and 24% of DDGS for a 63 days period. All experimental diets were isonitrogenous and isocaloric. Data were analyzed using the statistical software SAS University
Mycotoxins are secondary fungal metabolites that are detrimental to animal health and are a key concern to food and feed safety globally. The classic signs such as reduced feed intake and oral and intestinal lesions used as indicators of exposure often underestimate other costs of mycotoxicosis, including increased frequency and severity of disease via immunosuppression, inflammation, and modulation of the gastrointestinal environment. The objective of this study was to evaluate mycotoxin levels in corn and corn by-products from US 2019 harvest and compare those with previous years. Samples of US corn and corn by-products were analyzed utilizing liquid chromatography and tandem mass spectrometry (LC-MS/MS) for six major mycotoxin groups: aflatoxins (Afla), type A trichothecenes, type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN), and ochratoxin A. Mycotoxin quantity and co-occurrence data were analyzed by a Kruskal-Wallis test with a Dunn’s correction (Prism 7). For the 2019 harvest year, a high proportion of corn and corn by-products were found, at least one mycotoxin (96% and 100%, respectively), similar to what was observed in 2018 (99% and 95%, respectively). The occurrence in corn thus far is comparable with 2018 from 2% to 7% positive (P<0.05) and average contamination levels rising from 3 to 13ppb. Based on this year’s results, mycotoxins are present in the majority of corn and corn by-product in the US and should be considered as part of differential diagnosis when assessing issues on farm. Further research into understanding how mycotoxins encountered in the field contribute to subclinical production losses and their contributions to disease processes are essential for continuing success and advancements in the poultry industry.

**Key Words:** Co-product, corn distillers dried grains with solubles, egg quality, laying quails, performance

**221 Not Presented.**


A total of 240 male broilers were used in a 21-d experiment to study the effect of reference diet type on nitrogen corrected-apparent metabolizable energy (AMEn) of soybean meal (SBM) and canola meal (CM). The two reference diets were corn-SBM or corn-CM. To prepare experimental diets, graded levels of SBM or CM at 300 and 450 g/kg were added to each of the two basal diets to make a total of 10 diets. The experimental diets were fed to birds from d 14-21. Excreta were collected on d 20 and 21. The AMEn values were calculated using the difference or the regression methods for SBM using SBM- and CM-reference diets, and for CM using SBM- and CM-reference diets. The data for AMEn determined using the difference method were analyzed as 2×2×2 factorial to determine the effect of reference diet (corn-SBM and corn-CM), protein feedstuff (SBM, and CM), and substitution level (300 and 450g/kg) of assay feedstuff in the reference diets. There was significant (P < 0.05) three-way interaction between reference diet, feedstuff, and substitution level. For AMEn of SBM determined using CM reference diet, AMEn increased (P < 0.05) as the inclusion level of CM in the CM reference diet increased. The AMEn of CM decreased (P < 0.05) with increasing inclusion level of CM in the SBM-reference diet. Data on AMEn determined by the regression method were analyzed as a 2x2 factorial to determine the effect of the reference diet (SBM- or CM-based) or feedstuff (SBM or CM). There was no significant reference diet × feedstuff interaction. AMEn was greater (P < 0.01) when determined in a corn-CM reference diet compared with corn-SBM reference diet but there was no significant difference between AMEn of SBM and CM. For
224 Withdrawn.

225 Correlation between data on proximal composition and protein quality variables of commercial samples of heat processed soybeans analyzed by wet chemistry or estimated by NIRS technology. L. Cámara, L. Aguirre, A.F. de Juan, G. Fondevila, J. Ben Mabrouk, Gonzalo Mateos*, UPM, Madrid, Madrid, Spain.

Commercial samples (n = 22) of fullfat soybeans (FFSB) were collected at random from several locations in Europe and North Africa. The samples were analyzed for DM, ash, CP, ether extract (EE), crude fiber (CF), and phosphorus as well as for protein quality traits [protein dispersibility index (PDI), KOH solubility (KOH), and trypsin inhibitor activity (TIA)] by wet chemistry and near-infrared spectroscopy (NIRS) technology. Wet analyses were conducted using official methods by the same laboratory. NIRS values were obtained in a second laboratory using their own calibration data to evaluate the FFSB samples. The determined values of the FFSB samples by wet chemistry analyses varied from 88.1 to 94.0 for DM, 4.6 to 5.8 for ash, 32.3 to 40.3 for CP, 17.2 to 21.8 for EE, 5.4 to 8.6 for CF, 0.4 to 0.6 for total phosphorus, 6.3 to 22.0 for PDI, 72.4 to 92.9 for KOH, and 2.0 to 14.6 for TIA. The Pearson correlation (r) analyses (SAS Institute Inc., 1990) was used to study the relation between chemical composition of the FFSB determined by wet chemistry and values estimated by NIRS. P-values of less than 0.05 were considered significant. The correlation between both methods were high (r = 0.85) and very significant (P<0.001) for CP (r = 0.975) and DM (r = 0.940). The correlation was also high and very significant (P<0.001) for EE (r = 0.740), ash (r = 0.660), and KOH (r = 0.765) and still acceptable for phosphorus content (r = 0.511; P<0.01), PDI (r = 0.525; P<0.05), and TIA (r = 0.559; P<0.05). The correlation value between both methodologies for CF was found to be not significant (P>0.05). The low correlation between both methods observed for CF was unexpected and deserves a further evaluation of the methodology used for its determination in the lab. It is concluded that NIRS technology is a good alternative for estimation of the DM, CP, EE, ash, and phosphorus content of commercial fullfat soybeans, as well as for the evaluation of their PDI, KOH, and TIA.

Key Words: canola meal, soybean meal, reference diet type, metabolizable energy assay

Corn is a major component of feed and contributes significantly to the total dietary energy consumed by broilers. In feed formulation, it is therefore important to accurately estimate the nutritive value of corn. Previous research showed apparent metabolizable energy (AMEN) of corn varied due to differences in proximate composition, as well as intrinsic kernel factors such as kernel hardness, density, size, and vitreousness. However, current prediction equations used to estimate the AMEn of corn do not consider differences in digestibility arising from differences in kernel hardness or physiochemical structures. The objectives of this study were to determine which intrinsic kernel factors contribute to the observed variance in AMEn of corn samples and if these factors improved the accuracy of a model to predict the AMEn of corn. White (n=471) and yellow (n=639) corn samples from the 2015/2016 (n=338) and 2016/2017 (n=772) harvest seasons were collected from different regions in South Africa and analysed for moisture, protein, crude fat, milling index, grit yield all, AMEn, and hectolitre mass (L/kg) using near-infrared transmittance (NIT). The Pearson product moment correlation coefficient of all variables was determined using the multivariate analysis test. The relationship between AMEn and all parameters was analysed using the multiple regression model fit test. Factors were included in the final model using stepwise regression to minimise the Bayesian information criterion (BIC). Positive correlations were observed between AMEn and fat (r=0.63), protein (r=0.58), and moisture (r=0.32). A negative correlation between AMEn and corn starch (r=-0.60) can be explained by starch being negatively correlated with fat (r=-0.34) and protein (r=-0.85). Physical kernel properties that explained variance in AMEn included hectolitre mass (r=-0.47); milling index (r=0.55); and grit yield all (r=0.52). The final model selected was: AMEN=3589.8 + 37.59 * crude fat + 9.76 * crude protein - 30.32 * moisture + 0.3 * milling index. The R² of this model was 0.89 with a root mean square error (RMSE) of 8.95 kcal/kg. Results showed that variance in the AMEn of corn arose from differences in intuitive parameters such as proximate composition, as well as physical kernel properties. In addition to proximate...
composition, physical properties of the kernel should be considered when predicting AMEn for broilers.

Key Words: Corn, AMEn, broiler, Variation, prediction

227 Corn drying temperature, particle size, and amylase supplementation on live performance and nutrient utilization of Cobb 500 chickens. Gustavo Quintana-OspinaGS*, Hernan A. Cordova-Noboa1 Andres Ortiz1,2, Ylimar Matta1,2, Sebastian Hoyo1,2, Gherly D. Buitrago1,2, Juan D. Martinez1,2, Jonathan Yanquen1,2, Lorena Castellanos1,2, Jose O. Sorbara1, Edgar O. Oviedo-Rondon1, 1Prestige Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, United States, 2Facultad de Medicina Veterinaria y Zootecnia, Universidad del Tolima, Ibagué, Tolima, Colombia, 3DSM Nutritional Products, Kaiseraugst, Switzerland.

Corn-processing factors and enzyme supplementation may affect broiler live performance and nutrient utilization. This experiment evaluated grain drying temperature (DT), post-grinding particle size (PS), and exogenous amylase supplementation (AS) in yellow dent corn with hard endosperm. 12 dietary treatments resulted from a 2x2x3 factorial arrangement of two corn DT 35 and 120°C, two PS coarse (898 mm) and fine (338 mm), and three AS levels 0, 133, and 266 g/ton of Ronozyme® HiStarch with 8 replicates per treatment combination. A total of 480-d-old Cobb 500SF male chicks were randomly distributed in 96 cages (5 chicks/cage). BW and feed intake (FI) were recorded at 14 d, BW gain, and FCR adjusted for mortality were calculated, and fecal and ileal samples were collected at 14 and 16 d, respectively. The AMEn, total tract and apparent ileal digestibility (AID) of dry matter, CP, and starch were determined using titanium dioxide as an inert marker. Data were analyzed using a three-way ANOVA in a completely randomized block design. Mean separation was performed through Tukey’s or student’s t-test. On live performance, interaction effects (P<0.05) were observed in FI and FCR. Chicks fed coarse corn diets with 0 and 133 g/ton of amylase had a higher FI as compared to those supplemented with 266 g/ton. Chicks fed diets containing corn dried at both DT and with 266 g/ton of amylase were more efficient (P<0.05) than chicks fed diets with corn dried at 35°C without AS. Moreover, fine corn diets resulted in heavier chicks (P<0.05) with better BWG and better FCR. Meanwhile, chicks fed diets with 0 or 133 g/ton of AS ate more but had worst FCR. On digestibility parameters, interaction effects were observed on the AID of CP, starch (P<0.05), and fecal digestibility of CP (P=0.06). Ileal digestibility of protein was better in chickens fed diets without AS containing coarse corn dried at 35°C than at 120°C, and in fine corn dried at 120°C was better no AS than 266 g/ton of AS. However, in diets containing corn dried at 120°C, the AS at 266 g/ton improved the total tract CP retention by ~5% compared to non-supplemented diets. The digestibility of starch was 2.4% higher (P<0.001) in diets with corn dried at 35°C than in diets containing corn dried at 120°C regardless of the AS level. Finally, AS enhanced energy utilization (P<0.05) by 147 kcal on average as compared to chickens non-supplemented. In conclusion, grinding fine corn with hard endosperm improved young chicken live performance, the AS at 266 g/ton contributed to better FCR, and both AS levels improved energy utilization. On the other hand, low corn DT (35°C) and exogenous amylase improved protein and starch digestibility in chickens.

Key Words: drying temperature, particle size, amylase supplementation, live performance, nutrient digestibility


Particle size (PS) is known to play an important role in improvement of feed milling efficiency and animal growth. Institutions have developed different methods to analyze PS of feed ingredients and finished feeds to provide feed manufacturers and nutritionists with different tools to accurately determine PS. Therefore, 2 randomized complete block design experiments were conducted to evaluate different sieving methods to effectively determine PS of ground corn. In experiment 1, corn was ground using a hammer mill (15 × 38 Roskamp Champion) with a screen size of 11.11, 7.94, and 4.76 mm at 1,800, 1,350 and 900 rpm each, and 1.98 mm at 1,800 and 1,350 rpm. PS analysis was performed in these 11 different grinds in triplicate using the ANSI/ASABE S319.4 “Method of determining and expressing fineness of feed materials by sieving” 13-sieve method (ASABE). Experiment 2 had a 5 × 4 factorial treatment arrangement. Corn samples ground with a screen size of 11.11 mm at 1,350 rpm and 900 rpm, 7.94 and 4.76 mm at 1,350 rpm, and 1.98 mm at 1,800 rpm were analyzed. These 5 settings generated samples with mean PS of 1,562, 1,214, 1,051, 850 and 525 µm, respectively. Five replicate samples were analyzed using 4 different PS methods: 1) ASABE, 2) ASABE with addition of a powdered sieving flow agent (ASABEAG), 3) a 3-sieve hand-shaken method developed by Kansas State University for field testing of PS (KSU3S), and 4) a modification of the KSU3S method in which formulas from ASABE method were adapted for use with 3-sieves (AUM3S). Geometric standard deviation (SGW), surface area (SA) and particles per g (PPG) were also calculated in experiment 1 and with both ASABE methods and AUM3S in experiment 2. Data were analyzed using the GLIMMIX procedure of SAS (v9.4), least square means were separated with the PDIFF option, and considered different when P≤ 0.05. PS, SGW, SA and PPG values were different among the 11 settings evaluated in experiment 1 (P≤ 0.0001). Corn PS values differed among all methods evaluated in experiment 2 (P≤ 0.0001). Use of a sieving aid with ASABEAG reduced corn PS when compared to ASABE (P ≤ 0.0001). AU3MS produced higher PS values than KSU3S on all settings (P≤ 0.0001). Our results indicated that finer PS of corn resulted in reduced variability for SGW and increased PPG in ASABE, ASABEAG and AUM3S methods (P≤ 0.0001).
An increase was observed in SA values as PS values decreased when analyzing samples using both ASABE and AUM3S method but not with ASABE AG. Adjustments to the procedure of a PS analysis method can affect values obtained from one composite sample, thus it is important to understand how each method is related to another and that each facility uses one method consistently.

Key Words: particle size, hammer mill, sieving, corn

Assessment of the angle of repose as a method to measure the flowability of different particle sizes of ground corn using different sample amounts and box dimensions. Henry J. Flowers, Moses E. Chilenje, Marc R. Presume, Jorge L. Sandoval, Gerardo A. Abascal-Ponciano, Wilmer J. Pacheco, Charles W. Starkey, Poultry Science, Auburn University, Auburn, Alabama, United States.

Flowability is the ability of grains and powders to convey during discharge from transportation or storage containers. Flowability of grains is often influenced by their particle size (PS). Poor flowability is a continual source of frustration for many commercial producers, as it often reduces rate of feed delivery to animal feeders. Angle of repose (AOR), which is the angle formed between horizontal surface and slope of the heap produced, can be used to assess flowability of ground corn. Flowability is inversely proportional to AOR. The objectives were to assess flowability of 5 particle sizes of ground corn by measuring AOR as well as to determine the impact of box dimensions and sample amounts. The experiment had a 5 × 3 × 4 factorial treatment arrangement and samples were analyzed in triplicate. Corn was ground using a hammermill (Roskamp Champions Series 900-12) at 5 screen settings to generate samples with mean PS of 532, 834, 1,078, 1,237, and 1,620 µm. Three transparent boxes with respective dimensions: 30 cm high × 30 cm long × 2.5 cm wide (box 1), 30 cm high × 30 cm long × 5 cm wide (box 2) and 30 cm high × 60 cm long × 5 cm wide (box 3) were built. Four amounts of ground corn (100, 250, 500, and 1,000 g) were poured into each box using a funnel, then AOR was measured using a two-pair roller mill (Roskamp Champion Series 900-12) at 8 different roller mill settings (RMS): 3-2, 3-3, 4-3, 4-4, 5-4, 5-5, 6-4, and 6-5 (top-bottom roller pair setting, 50.8 µm to 152.4 µm). PS was analyzed in triplicate using a 13-sieve ANSI/ASABE S319.4 standard with sieving agent and an electric sieve shaker (ASABE), a hand-shaken 3-sieve field method developed by Kansas State University (KSU3S), and KSU3S without carnucles and rubber balls as sieving aids (γKSU3S). PS ranged from 534 to 3,012 µm from setting 3-2 through 6-6 when measured with ASABE. Modification of ASABE formulas were used to calculate PS using sieving weight data from the KSU3S (AUM3S) and γKSU3S (γAUM3S) methods. Data were analyzed using SAS V9.4 PROC GLIMMIX and means separated with PDIFF at P ≤ 0.05. The AUM3S method produced similar PS values to ASABE for PS 534 through 1,374 µm (P = 0.1465) and different values for PS 1,581 through 3,012 µm (P = 0.0150). Whereas AUM3S and γAUM3S produced similar PS values to ASABE for PS 534 through 771 µm (P = 0.2857) and different values for PS 1,375 through 2,323 µm for AUM3S (P < 0.0001) and 3,012 µm for γAUM3S (P < 0.0001). Field methods KSU3S and AUM3S produced similar PS values to γKSU3S and γAUM3S, respectively (P = 0.0734). Additionally, ASABE, AUM3S, and γAUM3S methods allow for calculation of geometric standard deviation (Sgw), surface area (cm² per g), and particles per g. All methods produced similar Sgw values for PS 534 µm (P = 0.1064). Additionally, AUM3S was similar to ASABE for PS 1,375 and 1,581 µm (P = 0.8379) but underestimated values for PS 2,482 µm and above (P < 0.0001). The AUM3S method produced similar surface area values to ASABE for PS 534 and 3,012 µm (P = 0.9958) and was different for all other PS (P = 0.0015). Whereas γAUM3S differed from ASABE for all PS (P = 0.0248) and was similar to AUM3S for PS 771, 1,375,
acid segregation was less apparent in IPQ feed with only regions of the commercial broiler house (P<0.05). Amino glycine, alanine, leucine, and lysine in the eight defined activities and concentrations of aspartate, glutamate, prominent in PPQ feed, demonstrated by varying phytase (P=0.1805). Phytase and amino acid segregation were 2. However, the P:F did not differ with IPQ feed across regions when considering PPQ feed (P=0.0016), starting at the hopper and region 8 ending at the feed line activity. All regions contained 24 feed pans, with region 1 defined regions per feed line and used to determine P:F, ratios (P:F). Pellets and fines were collected from eight using separate one-way ANOVA analyses. The P:F varied motor. Both PPQ and IPQ feed metrics were evaluated using a commercially formulated separator fitted with a #5 sieve to determine pellet-to-fine quality (IPQ) feeds. The PPQ feed (53% pellets: 47% fines) was augered through a 152-meter long commercial broiler house. A commercially formulated broiler finisher diet was manufactured using techniques to create either poor pellet quality (PPQ) or improved pellet quality improvements. More research to study on-farm nutrient segregation effects to bird performance is warranted.

Key Words: particle size, field method, roller mill, sieve, surface area

231 Withdrawn.

232 Improved pellet quality reduces on-farm nutrient segregation. Courtney Poholsky*, Dan Hofstetter, John W. Boney, Penn State University, Howard, Pennsylvania, United States.

There is reluctance to adopt feed manufacturing techniques to improve pellet quality (PQ) due to lack of association between PQ, bird performance, and overall economics. Literature suggests that feeding higher proportions of pellets contributes to improvements in production efficiency. Therefore, understanding how PQ affects on-farm nutrient segregation is paramount to adopting techniques to improve PQ. The objectives of this experiment were to understand how feed particles segregate in a feed line and to measure amino acid and phytase segregation throughout a commercial broiler house. A commercially formulated broiler finisher diet was manufactured using techniques to create either poor poor pellet quality (PPQ) or improved pellet quality (IPQ) feeds. The PPQ feed (53% pellets: 47% fines) was augered through a 152-meter long commercial broiler house containing four feed lines and 192 feed pans per feed line. Feed samples from the 768 feed pans were collected, labeled, and stored in individual feed bags. The IPQ feed (64% pellets: 36% fines) was handled in a similar manner. All feed (1,536 samples) was transported to Penn State University and passed through a modified particle size separator fitted with a #5 sieve to determine pellet-to-fine ratios (P:F). Pellets and fines were collected from eight defined regions per feed line and used to determine P:F, pellet durability, amino acid concentration, and phytase activity. All regions contained 24 feed pans, with region 1 starting at the hopper and region 8 ending at the feed line motor. Both PPQ and IPQ feed metrics were evaluated using separate one-way ANOVA analyses. The P:F varied across regions when considering PPQ feed (P=0.0016), where region 8 contained 10.4% fewer pellets than region 2. However, the P:F did not differ with IPQ feed (P=0.1805). Phytase and amino acid segregation were prominent in PPQ feed, demonstrated by varying phytase activities and concentrations of aspartate, glutamate, glycine, alanine, leucine, and lysine in the eight defined regions of the commercial broiler house (P<0.05). Amino acid segregation was less apparent in IPQ feed with only aspartate and glutamate concentrations varying across regions (P<0.05). Phytase segregation was not apparent in IPQ feed (P>0.05). These data suggest a more uniform presentation of nutrients at the feed pan after investing in pellet quality improvements. More research to study on-farm nutrient segregation effects to bird performance is warranted.

Key Words: pellet quality, nutrient segregation, pellet durability, amino acid, feed manufacturing


Near infrared reflectance spectroscopy (NIRS) is a reliable analytical tool; however, to obtain accurate results, samples must be handled carefully as ambient factors and physical properties of samples can alter estimations of nutrient concentrations of feed ingredients. Particle size (PS) is known to affect the ability of NIRS to accurately estimate nutrient composition of corn. The objective of this study was to evaluate the effect of PS on NIRS nutrient estimations of corn when analyzed using calibrations developed for analysis of ground or unground samples. Corn was milled using a hammer mill with different screen sizes and tip speeds to achieve 5 mean PS being these 525, 850, 1,051, 1,214, and 1,562 µm. Additionally, corn was ground using a rotor grinder (Retsch ZM 200) using 0.5 and 1.0-mm screens which served as control treatments for calibration for ground corn based on NIRS equipment company recommendations. Whole corn served as control treatment for calibration for analysis of unground corn. Replicates (n = 15) for each PS were analyzed in a Bruker MPA NIR spectrometer using either a calibration developed for analysis of ground samples (GCal) or a calibration developed for analysis of unground samples (UGCal) to predict moisture, crude protein (CP), crude fat (CFAT), crude fiber (CFIB) and starch content (SC). Data were analyzed using the GLIMMIX procedure of SAS V9.4. Least square means were separated using the PDIFF option and considered different when P ≤ 0.05. Coefficients of variation (CV) were calculated for each nutrient for both calibrations. Moisture content of samples with different PS analyzed with UGCal was consistently higher (P<0.0001) than samples analyzed with GCal. For CFAT content, differences were observed between PS and calibrations, samples analyzed with UGCal had higher (P < 0.0001) CFAT content estimations than those analyzed with GCal. Variation in CFIB content due to differences in PS was greater for UGCal (CV = 60.2%) than for GCal (CV = 15%). For UGCal CP content increased as PS increased (P < 0.0001). In contrast, for GCal CP content decreased as PS increased (P < 0.0001), except for whole corn in which CP was 1.2% higher than control treatments. Estimations of SC were consistently lower (P < 0.0001) in samples analyzed.
with GCal ranging from 50.16 to 71.99%, while SC content of samples analyzed with UGCcal ranged from 65.86 to 80.11%. In conclusion, analyzing samples with different PS than recommended for the NIRS calibration results in incorrect nutrient estimations in calibrations for analysis of ground corn as well as in calibrations for analysis of whole corn. This underscores the importance of proper sample handling when using NIRS technology.

**Key Words:** NIRS, calibration, particle size, corn, nutrient analysis
Dietary inclusion of the vitamin D3 (D3) metabolite, 25-hydroxycholecalciferol (25OHD3), has been shown to improve broiler muscle growth via a satellite cell-mediated muscle hypertrophic growth. To assess the effect of combined maternal and post-hatch dietary 25OHD3 inclusion on Pectoralis major (PM) muscle growth characteristics, a randomized complete block design experiment with the main effects of maternal diet (MDIET) and post-hatch diet (PDIET) arranged in a 2 × 2 factorial treatment structure was conducted. All diets were formulated to provide an equivalent of 5,000 IU of vitamin D per kg of feed. From 25 to 36 wk of age, commercial parent stock broiler breeder hens reared without 25OHD3 were provided 1 of 2 MDIET: 5,000 IU D3 (MCTL; ROVIMIX D3 500) or 2,240 IU of D3 + 2,760 IU of 25OHD3 per kg of feed (M25OHD3; 69 μg ROVIMIX HylD). The male broiler offspring (n = 400) hatched from eggs collected from 35 to 36 wk of age were reared in raised floor pens. Broilers were fed 1 of 2 PDIET in 2 phases (starter d 0 to 16; grower d 17 to 29): PCTL = 5,000 IU of D per kg of feed or P25OHD3 = 2,240 IU of D3 + 2,760 IU of 25OHD3. PM fiber density (P<0.015) on d 29. When combined with other work where 25OHD3 increased PM yield in broilers ≥ 40 d post-hatch, these results indicate that broilers receiving 25OHD3 in either the MDIET or PDIET had altered hypertrophic muscle growth prior to d 22 that ultimately led to reduced severity of WB by d 29.

**Key Words:** 25-hydroxycholecalciferol, vitamin D, maternal nutrition, broiler chicken, Wooden Breast myopathy
challenge, the expression of 1α-hydroxylase increased (P=0.025) in birds that received 25OHD₃ alone in comparison to all other in ovo-injected treatment groups. Also, the expression of 24-hydroxylase decreased (P=0.035) in birds that received 25OHD₃ in comparison to those injected with diluent or D₃ alone. Formation of the active form of the D₃ is facilitated by 1α-hydroxylase, whereas, 24-hydroxylase converts 25OHD₃ to the inactive form of D₃. Therefore, it was concluded that the in ovo injection of 2.4 μg of may increase the activity of D₃ in association with an increase in the expression of 1α-hydroxylase and a decrease the expression of 24-hydroxylase in Ross 708 broilers subjected to a coccidiosis challenge.

**Key Words:** 25-hydroxyvitamin D₃, Broilers, Coccidiosis, In ovo injection, Vitamin D₃ activity-related genes

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**Effects of the in ovo injection of vitamin D₃ and 25-hydroxyvitamin D₃ on the immune-related gene expression of broilers challenged with coccidiosis.** Saman Fatemi*, Katie E. Elliott, Abdul Mohssem Alqhtani, Ayoub Moussstaaid, Li Zhang, E. David Peebles, Poultry Science, Mississippi State University, Starkville, Mississippi, United States.

The expression of anti-inflammatory and pro-inflammatory genes has been shown to be improved by the use of supplemental dietary 25-hydroxyvitamin D₃ (25OHD₃) during a coccidiosis challenge. But these effects have not been investigated in response to the in ovo injection of either vitamin D₃ (D₃) or 25OHD₃. Therefore, the objectives of this study were to determine the effects of the in ovo administration of D₃ and 25OHD₃ on the expression of the pro-inflammatory and anti-inflammatory genes of broilers challenged with coccidiosis. Live embryonated Ross 708 broiler hatching eggs were randomly assigned to one of the following 5 in ovo injection treatments at 18 d of incubation (d oi): 1) non-injected; 2) diluent; diluent containing either 3) 2.4 μg D₃, 4) 2.4 μg 25OHD₃, or 5) 2.4 μg D₃ + 2.4 μg 25OHD₃. A 50 μL solution volume was injected into each egg using an Inovject multi-egg injector. At hatch, 4 male chicks were randomly assigned to each of 40 battery cages in each of 2 rooms with 8 replicate pens assigned to each treatment group (320 total birds across both rooms). The experimental design was a randomized complete block and room was the blocking factor. Only in ovo-injected treatments were challenged with a 20x dosage of live coccidial vaccine at 14 d of age (d o a). At 14 and 28 d o a, jejunum samples were collected from 1 bird from each replicate pen for gene expression analysis. Expression of 2 house-keeping genes (β-Actin and GAPDH) and 3 immune-related genes (IL-10, TLR-4, and MyD88) were performed using Real-Time PCR at 14 and 28 d o a. Main effect of injection treatments was analyzed using one-way ANOVA, with significance set at P≤0.05. No significant differences were observed among treatments for all genes before challenge. However, at 2 wk post-challenge, the expression of IL-10 increased (P=0.042) in birds that received 25OHD₃ alone in comparison to those in ovo-injected with D₃ + 25OHD₃ or diluent. Dietary 25OHD₃ has been shown to increase the expression of IL-10 in birds challenged with coccidiosis. Additionally, increased expression of IL-10 is associated with reduced inflammation and increased BW gain of broilers in response to a coccidiosis infection. Therefore, it was concluded that the in ovo injection of 2.4 μg of 25OHD₃ may reduce inflammation in association with an increase in the expression of IL-10 in Ross 708 broilers subjected to a coccidiosis challenge.

**Key Words:** 25-hydroxyvitamin D₃, Broilers, Coccidiosis, immune-related genes, in ovo injection

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**237 The Effect of Dietary Cadmium on Production Performance of Laying Hens and Its Accumulation in Tissues.** Caim Wu*, Juan Zhang, Guangmang Liu, Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu, China.

Cadmium (Cd) is one of the most commonly toxic heavy metal pollutants, it occurs everywhere in the environment, and is known to pose threats to human and animal health by spreading through food, water, soil even ambient air in low concentration. The objective of this trial is to investigate the effect of dietary Cd on production performance of laying hens and its accumulation in tissues. A total of 150 (40-wk-old) Lohmann pink-shell laying hens were assigned to a completely randomized design with five treatments (0, 10, 30, 50, 70 mg/kg Cd (cadmium chloride) of diet) and three replicates of ten birds each. Laying hens were fed with a corn-soybean meal basal diet and had ad libitum access to feed and water. Production performance indexes were measured every week. On day 0, 5, 10, 17, 26, 35, 41, 46, 51, 56, eggs were collected and separated for yolk and egg white samples. On day 57, three birds per replicate were randomly selected and euthanized for tissues collection. The Cd residues were analyzed by high resolution-continuum source flame atomic absorption spectrometry. Data were analyzed using the General Linear Model (GLM) procedure and PROC MIXED (SAS 9.4) and difference between treatments means were compared by Tukey’s multiple Range Test. A P value less than 0.05 was considered statistically significant. The results were as follows: (1) Compared with control group, Cd of 10 mg/kg group increased the average daily feed intake (ADF1), and Cd of other treatment groups reduced gradually ADFI with Cd dose increasing, the ADFI of 70 mg/kg Cd treatment group was significantly lower than that of the control group (P<0.05). The laying rate decreased with Cd dose increasing, and there was no significant difference among 10 to 50 mg/kg Cd treatment groups, but that of the 70 mg/kg Cd treatment group had a significant decrease compared with others treatments (P<0.05). Other production performance indexes didn’t have significant difference among treatments (P>0.05). (2) The accumulation of dietary Cd in different tissues followed the order: kidney and liver > small intestine and the reproductive organ (ovary > oviduct) > heart and muscle > cerebrum > egg yolk > egg white, with a dose-dependent
pattern. Therefore, dietary Cd of low dose maybe improve production performance of laying hens, dietary Cd of high dose decrease production performance, and cumulative Cd in different tissues could increasing their susceptibility to disease.

**Key Words:** cadmium, laying hens, production performance, accumulation

238 Effect of limestone particle size and potassium supplementation on live performance and blood physiology of male broiler chickens. Dinabandhu Joardar*, Kimberly Livingston, Frank Edens, Rasha Qudsieh, Matthew Livingston, John Brake, Prestage Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, United States.

The experiment investigated the effects of limestone particle size and dietary potassium (K) on live performance, blood physiology, and muscle myopathies in broilers raised to 35 d of age. A total of 384 Ross male broilers were placed in 24 floor pens and fed four diets in starter (0-16 d of age) and grower (17-35 d of age). Diets were formulated by combining two limestone particle sizes (fine: 0.2 mm and coarse: 0.9 mm), and two dietary K levels (0% basal K (K−) or 0.2% added K (K+)) as potassium carbonate to complete the 2x2 factorial arrangement. There were 6 replicate pens per factorial treatment. Feed intake (FI), BW, and BWG were measured from 1-33 d of age, while blood physiology, woddy breast (WB) and white striping (WS) scores were measured at 35 d of age. Blood physiology was measured on birds of similar BW to reduce variation in physiological response caused by variation in BW. Score scale from 1-4 was used for both WB and WS (1=normal to 4=severe) and estimated for all 16 birds per pen. Data were analyzed as 2-way ANOVA. There were no 2-way interactions. The K+ dietary treatment reduced (P < 0.05) FI by 3.5% and 3.0%, and BWG by 4.0% and 3.0% when compared to K− at starter and grower periods, respectively. The K+ dietary treatment decreased (P < 0.05) blood Na (mmol/L), blood glucose (mg/dL), ionized blood Ca (mg/dL), TCO2 (mmol/L), blood HCO3 (mmol/L), and base excess in extracellular fluid (mmol/L) when compared to K− birds of similar BW at 35 d of age (to establish a similar . Fine limestone diets tended (P = 0.08) to reduce WB scores (3.0 vs. 2.59) when compared to coarse limestone diets at 35 d of age. This study showed that using 0.2% of K as potassium carbonate did not negatively affect mortality-adjusted FCR even though FI and BWG was reduced. Furthermore, fine limestone has the potential to reduce WB in breast muscles; however, further research is needed to confirm these outcomes.

**Key Words:** Limestone particle size, potassium, blood physiology, myopathies

239 A coordinated dysregulation of candidate genes signs a deteriorated Ca/P balance in ageing laying hens. Audrey Gloux1, Nathalie Le Roy1, Nathalie Même2, Marie-Liesse Piketty1, Dominique Prie2, Gaelle Benzoni2, Joel Gautron1, Yves Nys1, Agnes Narcy1, Michel J. Duclos*1, BOA INRAE Université de Tours, Nouzilly, France, 1ADM Animal Nutrition, Saint Nolff, France, 1Institut Necker-Enfants Malades, INSERM U1151-CNRS UMR8253, Paris, France.

Numerous studies reported alterations of bone strength in hen’s flocks at the end of lay and qualified it as an osteoporotic syndrome. This must result from the priority of the Ca supply to the shell gland for ensuring the calcification of the eggshell, even if at the expense of the bone. This suggest an altered Ca metabolism in the extra uterine compartments of the hen, the underlying mechanisms of which remain unknown. The present study aimed to describe the effect of ageing in laying hens on the expression of candidate genes in the tissues involved in Ca retention and its regulation. Individually housed hens of a brown egg line aged 23 or 90 weeks, were submitted to blood collection prior to euthanasia at three stages of the ovulatory cycle (0-1 h, 9-10 h and 18-19 h post ovulation). Plasma samples were used for the measure of plasma iP, and for 25(OH)D3 and 1.25(OH)2D3 immunoassay. Tissue samples were collected from parathyroid gland (PG), liver, jejunum, medullary bone (MB) and kidney for a quantitative study of candidate gene expression (46 candidate genes and 9 housekeeping genes) by high-throughput real-time RTPCR, (BMK-M-96.96, Fluidigm®). The data were analyzed using a linear model by a robust regression (R Package MASS version 7.3-51.1), to test the effects of the main factors PO stage, age and their interaction. Subsequent pairwise comparisons were performed using the least-square means (LSMeans) method (R Package emmeans version 1.3.0) and a Tukey adjustment. A strong challenge to Ca homeostasis was suggested in aged hens, which showed decreased circulating 1.25(OH)2D3 levels (p < 0.01). In the jejunum, this was accompanied by the decreased expression of 5 candidate genes involved in Ca transport (p < 0.01) and of Vitamin D Receptor (VDR, p < 0.0001). In the kidney, a decreased expression of the transient receptor potential channel subfamily V member 5 (TRPV5) involved in Ca reabsorption was observed (p < 0.05). This could exacerbate bone resorption and impair bone accretion, as attested by a higher expression of the Carbonic Anhydrase 2 (CA2, p < 0.05) gene and a lower expression of collagen type I alpha 1 chain (COL1A1, p < 0.05) in the MB. A markedly increased expression of Fibroblast Growth Factor 23 (FGF23, p < 0.05) in the MB was also observed at 90 week old, which likely contributed to the decreased plasma levels of 1.25(OH)2D3 and the altered expression of target genes under its regulation. Our data highlights some coordinated molecular mechanisms underlying the osteoporotic syndrome previously documented in aged laying hens, and suggest FGF23 as a central player in this context.

**Key Words:** laying hen, calcium metabolism, endocrinology

240 Effects of dietary Ca and microbial phytase on...
The aim of this study was to evaluate the effects of microbial phytase (PHY), dietary calcium (Ca) and zinc (Zn) sources, i.e. oxide (ZnO; HiZox®) vs sulfate (ZnSO4) on performance, bone mineralization and Zn concentration in tibia and plasma in broiler chickens. Eight diets were fed to 144 male broilers (Ross 308) from 8 to 21 d according to a 2x2x2 factorial arrangement: 2 levels of Ca (6 and 10 g/kg), 2 sources of Zn (ZnO and ZnSO4) and 2 levels of PHY (0 and 750 FTU/kg). Animals were randomly assigned in the treatments and placed in individual cages. Diets contained 27 ppm native Zn and 23 ppm supplemented Zn as ZnO or ZnSO4 with a non-PP level of 4.5 or 3.2 g/kg (0 or 750 FTU/kg, respectively) and a PP content of 2.3 g/kg. Data was subjected to a 2x2x2 ANOVA analysis. Dietary Ca content was slightly lower than expected in the low Ca diets (5.3 vs 6.0 g/kg). Body weight (BW) gain and feed efficiency were positively affected by ZnO compared to ZnSO4 (+27g, P<0.01, +0.03 points, P<0.001, respectively). The high Ca level improved feed intake (+32g, P<0.01) and final BW (+25g, P<0.05). A lower gain was observed when PHY was supplemented to low-Ca diets probably due to a Ca deficiency (Ca* PHY, P<0.05). Tibia weight, ash and length were positively affected by ZnO compared with ZnSO4 (P<0.05, P=0.062 and P<0.01) and is linked to the significant effect of ZnO on growth. High Ca affected positively tibia weight, ash weight and content and breaking strength (P<0.0001) illustrating the requirement in Ca for bone growth and mineralisation. The skeleton being a main site of storage of Zn, high Ca improved tibia Zn content and deposition whatever the Zn source (+4.9 mg/kgDM, P<0.01, +38µg, P<0.0001). PHY supplementation increased tibia Zn deposition (+53µg, P<0.0001) as well as tibia Zn content dependently of the Zn source (PHY *Zn source, P<0.05) with an improvement of 21 and 15 mg/kgDM in birds fed ZnSO4 and ZnO, respectively. Plasma Zn, a short-term indicator of Zn availability, also increased with PHY supplementation (P<0.001). There was a trend for the interaction PHY*Zn source (P=0.067) as PHY increased plasma Zn only in birds fed ZnO. The effect of PHY on Zn availability seems to depend on the Zn source but also on the response criteria. Dynamics of Zn absorption and deposition are different. Zn from ZnO is quickly absorbed compared with sulfate but bone deposition seems more gradual, which would explain the differential response observed between plasma and bone Zn. ZnO allowed better performance response of broilers. Dietary Ca level affects Zn utilization independently of the Zn source whereas the effect of PHY depends on it.

Key Words: Zinc, Phytase, Broiler, Calcium

241 Dimensional profile of broiler tibias is affected by dietary zinc source. Marquisha Paul*, Anthony Pescatore, Michael Ford, Tuoying Ao, Karl Dawson, Alltech-

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Previous results from our research group indicate that breeder brooder nutrition, specifically dietary microalgae (FORPLUS™, Alltech, Inc.; source of docosahexaenoic acid) and organic Zn source (Bioplex® Zn, Alltech, Inc.; chelated Zn-proteinate) can have a direct impact at time of hatch on offspring skeletal leg development including hatch growth plate morphology and dimensional characteristics. Little information is available on the interaction effects of breeder and offspring nutrition on skeletal development post-hatch. This study was conducted to determine the effects of breeder breeder diet and offspring dietary Zn source (Bioplex® Zn vs. ZnO; ZnO technical grade, Fisher Scientific) on offspring tibia dimensional profile. An RCB design with a 4x2 factorial treatment structure consisting of 4 breeder diets and 2 offspring dietary Zn sources was used for this study. A total of 240 Cobb 500 breeder hens and 20 roosters were randomly assigned to 4 diets: 1) corn-soybean meal (CSM) diet + 40 mg ZnO/kg diet, 2) diet 1 + 2% FORPLUS™, 3) CSM diet + 40 mg Bioplex® Zn/kg diet, 4) diet 3 + 2% FORPLUS™. Breeders were raised in floor pens with ad libitum access to water and nest boxes, 15L: 9D lighting, and controlled access to feed. Fertile eggs were collected and set at 30-31 wk of lay. At hatch, 128 straight run broiler chicks from each breeder diet were randomly assigned to cages with a CSM diet + 2% FORPLUS™. Breeders were raised in floor pens with ad libitum access to water and nest boxes, 15L: 9D lighting, and controlled access to feed. Fertile eggs were collected and set at 30-31 wk of lay. At hatch, 128 straight run broiler chicks from each breeder diet were randomly assigned to cages with a CSM diet + 40 mg ZnO or Bioplex® Zn/kg diet. This resulted in 8 replicate cages per treatment with 8 chicks per cage. Broilers were provided ad libitum access to feed and water. At 3 wk of age, broiler tibia dimensional profiles were determined using a VolScan Profiler (Stable Micro Systems, Surrey, England). Data were subjected to two-way ANOVA (GLM procedure of SAS v9.4) with means separated using Fisher’s LSD test. No interaction of breeder diet and offspring Zn source on tibia dimensional profile was observed (P>0.05). Bioplex® Zn in the broiler diet increased (P<0.05) tibia shaft width and circumference compared to ZnO. Bioplex® Zn in the broiler diet tended to increase (P<0.10) tibia volume, weight, and surface area compared to ZnO, while tibia length tended to be influenced (P<0.10) by Bioplex® Zn in the breeder diet. In conclusion, dietary supplementation of Bioplex® Zn in broiler diets increased tibia dimensions had the greatest impact on the dimensional profile of broiler tibias a 3 wk of age.

Key Words: Zinc, Skeletal leg development, VolScan Profiler, broiler, broiler breeder

242 The relative bioavailability of inorganic and organic zinc sources for weight gain in broiler chickens. Mohammad Pilevar*, Oluyinka Olukosi, Poultry Science, University of Georgia, Athens, Georgia, United States.

A 21-day study was conducted to determine the relative bioavailability of zinc (Zn) from hydroxychloride as inorganic Zn source and glycine and lysine chelated-Zn as organic sources, using zinc sulphate hepta-hydrate
(ZnSO₄.7H₂O) as the reference Zn source. A total of 288 male broiler chicks (Cobb 500) were allocated into 9 treatments, each with 8 replicates and 4 birds per replicate in a Randomized Complete Block Design. The chicks received a corn-soybean meal diet on day 0 to 3 followed by a Zn-free cornstarch-soybean protein concentrate purified diet from day 3 to 7 to deplete the birds Zn stores. The purified diet was supplemented with two levels of each of the 4 Zn sources (ZnSO₄.7H₂O, hydroxychloride Zn, Zn-Glycinate, and Zn-Lysine) to provide 50 mg/kg or 100 mg/kg Zn in experimental diets which were fed from day 7 to 21. The basal diet was Zn-free and used as a control diet. The relative bioavailability of Zn, based on weight gain, was assessed using slope-ratio assay using ZnSO₄.7H₂O as the standard Zn source. Body weight and feed intake data were collected on days 7 and 21 of experiment. On day 21, two birds per cage were selected and euthanized for tibia and toes sampling. Also, blood was collected via the wing vein in two birds per replicate to measure creatine kinase activity in serum samples. Body weight gain was regressed against Zn intake, and the ratio of the slopes used to calculate the relative bioavailability of Zn for weight gain. The relative bioavailability values were 107%, 76% and 127% for hydroxychloride, Glycinate and Zn-Lysine sources, relative to the Zn sulfate. There was quadratic increase (P < 0.05) in % tibia and toe ash of chicks fed Zn-Lysine. Creatine kinase activity decreased (P < 0.05) both linearly and quadratically in response to ZnSO₄.7H₂O but increased in Zn-Lysine sources diet. Additional investigations are being conducted to elucidate the modes of action for the differences in response observed with the different Zn sources.

Key Words: Zinc, inorganic, organic, bioavailability, chicks

243 Effects of the in ovo injection of L-ascorbic acid (AA) on the serum AA concentrations and hatchability of broiler embryos. Ayoub Mousstaaid GS*, Saman Fatemi, Katie E. Elliott, Abdul Mohssen Alqhtani, David Peebles, Poultry Science, Mississippi State University, Starkville, Mississippi, United States.

Effects of the in ovo injection of various concentrations of L-ascorbic acid (AA) on the serum AA concentrations and hatchability of broiler embryos was determined. A total of 960 Ross 708 broiler hatching eggs collected at peak egg production were randomly allocated to 4 treatment groups: a non-injected control, saline-injected control, or saline containing 12 or 25 mg of AA. At 18 d of incubation (doin), fresh sterile saline (0.85%; 100 μL) alone or containing different levels of AA were injected into the amnion using a Zoetis Inovoject-M multi-egg injector. Each of the 4 treatment groups were represented on each of 8 replicate tray levels in a Natureform incubator. Eggs were candled at 9 and 18 doi, percentage egg weight loss was calculated between 0 and 12, and 12 and 18 doi, and hatchability and hatchling BW were determined at hatch (21 doi). Hatchery residue analysis was performed after candling to assess embryonic mortality. Blood samples were collected from broiler embryos at 19 and 21 doi, and were stored at -20 °C until serum AA concentrations were determined using ELISA. A randomized complete block experimental design was employed, and data was analyzed using SAS 9.4. Serum AA concentrations of embryos at 19 and 21 doi were not significantly affected by the 12 and 25 mg in ovo-injected dosages of AA. However, across treatment, serum AA concentrations decreased between 19 and 21 doi. At 21 doi, hatchability and hatchling BW were also not significantly affected by treatment. Nevertheless, embryos in the non-injected control group exhibited a significantly higher rate of mortality (P ≤ 0.05) on d of hatch (21 doi) in comparison to all other treatment groups. These results suggest that the in ovo injection of 12 and 25 mg of AA does not significantly increase the serum AA concentrations of broiler embryos and consequently does not adversely affect subsequent hatchability or hatchling BW.

Key Words: L-ascorbic acid, in ovo injection, hatchability, concentration, serum
Horizontal transmission of *Salmonella* Enteritidis and *Salmonella* Kentucky 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, 1US National Poultry Research Center, USDA-ARS, Athens, Georgia, United States, 2Poultry Science, North Carolina State University, Raleigh, North Carolina, United States, 3Animal Science, Purdue University, West Lafayette, Indiana, United States.

The transmission of *Salmonella* infections to humans via contaminated eggs is an international public health concern. *S.* Enteritidis is deposited inside eggs after colonizing reproductive tissues of infected hens. Diverse housing facility characteristics and flock management practices can influence *Salmonella* persistence and transmission in poultry flocks, but the full food safety consequences of different housing system options for egg-laying hens remain unresolved. The present study assessed the horizontal transmission of infection and invasion of internal organs after experimental *S.* Enteritidis and *S.* Kentucky 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. 24 hens in each room were orally inoculated with 6.5 × 10⁷ cfu of 2-strain mixtures of either *S.* Enteritidis or *S.* Kentucky (2 groups each). At 6 d and 12 d post-inoculation, 12 inoculated and 24 contact-exposed hens in each room were euthanized and samples of liver, spleen, ovary, oviduct, and intestinal tract were removed for bacteriologic culturing. All orally inoculated hens were positive for intestinal colonization by *S.* Enteritidis at 6 d post-infection and 70.8% of contact-exposed hens had become colonized by 12 d post-infection. *S.* Enteritidis was isolated from 100% of livers and 50.0% of ovaries from inoculated birds at 6 d post-inoculation and from 41.7% of livers and 10.4% of ovaries from contact-exposed birds at 12 d. The majority of both orally inoculated and contact-exposed hens were positive for intestinal colonization by *S.* Kentucky at 6 d post-infection, but *S.* Kentucky was found in other internal organs of both inoculated and contact-exposed hens significantly (*P* < 0.05 in Fisher’s exact test) less often than *S.* Enteritidis at both sampling intervals. These results indicate that *Salmonella* infection can spread rapidly and extensively among hens in cage-free indoor housing, including a high frequency of internal organ involvement for invasive serovars such as *S.* Enteritidis.

**Key Words:** Salmonella Enteritidis, Salmonella Kentucky, laying hens, internal organs, cage-free housing

The efficacy of the aflatoxin B₁-binder Mycofix® Secure demonstrated by serum and feces biomarkers of exposure. Nada Jurisic1, Elisavet Kunz-Vekiri1,2, Heidi Schwartz-Zimmermann1, Justin Fowler1, Wulf Dieter Moll1, Franz Berthiller2, Wolfgang Schweiger*1, BIOMIN Research Center, Tulln, Austria, 2Department for Agrobiotechnology, University of Natural Resources and Life Sciences, Vienna, Austria, 3Department of Poultry Science, University of Georgia, Athens, Georgia, United States.

The toxic carcinogenic fungal secondary metabolite aflatoxin B₁ (AfB₁) is a frequent contaminant in feed. Young chicks are particularly sensitive to continued exposure. The most reliable means to reduce AfB₁ levels are silicate-based binders that limit the bioavailability of AfB₁ for metabolic activation in the liver. Traditionally, the efficacy of such binders is inferred from performance and inflammatory response data. Instead, we report on the reduced abundance of the AfB₁-adduct to lysine (AfB₁-Lys) from serum albumin as a persistent and stable indicator of liver-activated AfB₁ biomarker that is unaffected by the presence of an AfB₁-binder. These data are complemented by the respective biomarker and metabolite in excreta AfB₁-N⁷-guanine (AfB₁-Gua). We have performed a 21-day animal trial starting with eight pens of each seven one-day-old chicks for each of six experimental groups. These included feed regimen with three aflatoxin levels (0, 20 and 500 µg/kg) with or without addition of the Mycofix® Secure bentonite binder (BIOMIN, Austria). Serum and excreta samples were collected weekly on a per pen or per bird basis. We developed and validated an HPLC-MS/MS method for the determination of aflatoxins B₁, B₂, G₁, G₂, M₁, P₁, Q₁ and the AfB₁-Gua in excreta and AfB₁-Lys in serum. AfB₁-Lys was synthesized via chemical epoxidation and incubation with BOC-lysine followed by deprotection with trifluoroacetic acid. Serum albumin levels were checked and samples were digested with Pronase to release AfB₁-Lys. Samples were measured after dilution, centrifugation, evaporation of the supernatant and reconstitution in solvent without additional clean-up steps. AfB₁ metabolites were detectable in excreta at all time points but only in samples from chicks exposed to the higher AfB₁ level. Without added binder AfB₁ levels in excreta increased from 4.5 to 13.5 ng/g dry matter in time, while AfB₁-Gua levels remained at around 1 ng/g. In contrast average AfB₁-Gua levels in presence of the binder dropped below the LOQ of 0.45 ng/g, while AfB₁ levels averaged between 112 and 132 ng/g. These high AfB₁ levels are recovered only under extraction conditions using +80% organic solvent that lead to desorption of AfB₁ from the bentonite binder. The up to 10x higher efflux of AfB₁ via feces is reflected by AfB₁-Lys levels in serum: The biomarker was detected in non-binder samples at the 14 and 21 day time points with in average 3.4 and 3.2 ng/mL (LOQ: 0.25 ng/mL). The respective binder-samples averaged at 2.1 and 1.8 ng/mL, resulting in an overall reduction of AfB₁-Lys of 40%.

**Key Words:** aflatoxin, mycotoxin, binder, biomarker
246 Gut-derived serotonin modulates \textit{Campylobacter jejuni} colonization \textit{in vitro}. Sandip Shrestha\textsuperscript{1}, Basanta Wagle\textsuperscript{1}, Rohana Liyanage\textsuperscript{2}, Annie M. Donoghue\textsuperscript{1}, Joshua M. Lyte*\textsuperscript{1}, \textsuperscript{1}USDA-ARS Poultry Production and Product Safety Research Unit, Fayetteville, Arkansas, United States, \textsuperscript{2}Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States.

**Objective:** \textit{Campylobacter jejuni} colonization of the chicken intestine is a major source of contamination of poultry products during processing. Neuroendocrine crosstalk between host and microbe may play a critical role in enteric colonization. Serotonin (5-hydroxytryptamine; 5-HT), is present in the gut lumen, yet its role in modulating growth and colonization of \textit{C. jejuni} is unknown. We therefore sought to determine the effect of serotonin on \textit{C. jejuni} growth and colonization \textit{in vitro}. **Experimental design:** The dose-dependent effect of 5-HT on \textit{C. jejuni} growth was determined. Next, it was determined how a sub-inhibitory concentration of 5-HT affected \textit{C. jejuni} protein expression. 5-HT caused the down-regulation of proteins essential in \textit{C. jejuni} colonization, therefore we assessed whether 5-HT affected \textit{C. jejuni} colonization in the HT29MTX-E12 mucus secreting polarized colon epithelial model \textit{in vitro}. **Materials and Methods:** \textit{C. jejuni} isolated from broiler chickens was inoculated into \textit{Campylobacter} enrichment broth in the presence of different concentrations of 5-HT based on prior published reports (0, 0.01, 0.1, 1, and 6.25mM; n=6/replicates/treatment), and incubated for 24 h at 42°C in a microaerophilic atmosphere. Growth was determined by plate enumeration. The effect of 5-HT on the \textit{C. jejuni} proteome was investigated using liquid chromatography tandem mass spectrometry (LC-MS/MS). The effects of 5-HT on \textit{C. jejuni} adhesion and invasion of HT29MTX-E12 cells were determined following washing of the monolayer and counting adherent bacteria. **Statement of statistical analyses:** Data were analyzed by ANOVA with Tukey’s posthoc test. For proteomic analysis, Scaffold Proteome Software was used to analyze MASCOT (Matrix Science) search results from uniprot \textit{C. jejuni} protein database and differentially expressed proteins between treated and untreated samples were determined using Student’s \textit{t}-test. **Results:** The sub-inhibitory concentration of 5-HT was 1 mM, and a dose dependent inhibition was observed at 6.25 mM. LC-MS/MS revealed 5-HT significantly (p<0.05) down-regulated the expression of bacterial proteins essential in colonization and carbohydrate metabolism. The adhesion of \textit{C. jejuni} to HT29MTX-E12 cells was significantly reduced (p<0.05); however, invasion was not different when compared to controls (p>0.05). **Conclusions:** 5-HT, a neurotransmitter produced in the gut, reduced the ability of \textit{C. jejuni} to colonize a mucus-secreting polarized colonic epithelium \textit{in vitro}. Further studies are required to understand the implications of these findings for the control of \textit{C. jejuni} enteric colonization \textit{in vivo} and to determine the mechanisms by which 5-HT modulates \textit{C. jejuni} colonization.

247 Recovery of \textit{Salmonella Enteritidis} and \textit{Campylobacter Coli} from inoculated and incubated hatching eggs. Caitlin Harris\textsuperscript{(a,b,i)}, L. N. Bartenfeld Josselson\textsuperscript{2}, R. J. Buhr\textsuperscript{2}, \textsuperscript{1}Poultry Science, University of Georgia, Athens, Georgia, United States, \textsuperscript{2}USDA-ARS, Athens, Georgia, United States.

Vertical transmission of \textit{Salmonella} from hen to offspring through the egg has been documented, but the data for \textit{Campylobacter} is lacking. \textit{Salmonella} and \textit{Campylobacter} spp. are important human foodborne pathogens and increased knowledge on egg transmission would be beneficial for intervention strategies. The objective was to develop a \textit{S. enteritidis} (SE) and \textit{C. coli} (CC) inoculation protocol for hatching eggs and then recover the bacteria from embryos and egg contents during incubation. Three experiments determined inoculum levels for SE (exp 1), inoculum levels for CC (exp 2), and recovery at 5 and 15 (exp 3). For all experiments, SPF white Leghorn eggs were collected 2-3 d prior to inoculation. For exp 1, there were 6 SE treatments (n=12): 1.22x10\textsuperscript{3}, 1.40x10\textsuperscript{5}, or 2.05x10\textsuperscript{7} CFU yolk or albumen injected. For exp 2, there were 6 CC treatments (n=12): 1.50x10\textsuperscript{1}, 4.50x10\textsuperscript{1}, or 3.90x10\textsuperscript{2} yolk or albumen injected. For exp 3, there were 4 treatments (n=22): SE 2.52x10\textsuperscript{3} CFU yolk or albumen injected and CC 3.30x10\textsuperscript{2} CFU yolk or albumen injected. For all inoculations, a hole along the equator of the egg was ground and inoculum was injected thru the shell membrane. Two eggs per treatment were sampled on d 0 to confirm inoculation. Eggs were incubated at 37.5°C and 54% relative humidity. On sampling days, embryos (when present) were aseptically removed from each egg and sampled separately from egg contents. Direct and enriched plating methods were performed on all sampling days to determine if egg contents or embryos were positive/negative. Kruskal-Wallis method was used to determine significance (p≤0.05) and Dunn method was used for mean comparisons. For exp 1, there were no significant differences for SE recovery at d 2 for egg contents and d 5 for embryos. There were significant differences (p<0.0001) for egg contents recovery at d 5, with the yolk inoculations for all levels having 100%, while the albumen inoculations had a recovery of 0 – 20%. For exp 2, there was a significant difference in CC recovery from egg contents at d 2 (p=0.0360), with 100% recovery for albumen 3.90x10\textsuperscript{2} CFU inoculation and 0 – 60% recovery for all other treatments. At d 5, there were no significant differences in CC recovery. For exp 3, recovery from egg contents at d 5 via yolk inoculation was 100% for SE and 90% for CC recovery. This was significantly higher (p=0.0050, 0.0087) compared to 40% for SE and 30% for CC for albumen inoculated. At d 15, there were no significant differences for SE, but CC egg contents were 100% positive for yolk inoculations compared to 50% for albumen (p=0.0137). These results show that there is potential for \textit{C. coli} to persist in hatching.
eggs similar to *S. enteritidis* and be recovered from incubated eggs.

**Key Words:** Salmonella, Campylobacter, vertical transmission, incubation, inoculation

### 248 Whole-genome sequence analysis of multidrug-resistant avian pathogenic *Escherichia coli* (APEC) MS1170 isolated from broiler.

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Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis, one of the leading causes of mortality and morbidity, leading to significant economic losses in the poultry industry. This study aimed to characterize the genome sequence of a multidrug-resistant (MDR) APEC strain isolated from a broiler chicken. The strain APEC MS1170 was isolated from the broiler yolksac of a 14d old broiler. Antimicrobial susceptibility testing was performed using the Sensititre system with 14 different antibiotics (YCMV4AGNF) and the automated ARIS 2X system (Thermo Scientific). Interpretation of results were performed according to the Clinical and Laboratory Standards Institute guidelines. Genomic DNA was extracted from the bacterial culture after overnight incubation in LB broth at 37 °C using the GeneJET Genomic DNA Purification Kit (Thermo Scientific). Whole-genome sequencing was performed by using both the long-read sequencing approach with a Nanopore GridION sequencer (Oxford Nanopore Technologies, Oxford, UK) and the short-read sequencing approach using an Illumina HiSeq X-Ten sequencer (Illumina, San Diego, CA, USA) allowing for a complete scaffold of the genome to be obtained as well as an accurate sequence. The genome sequence was produced with Canu v1.9 using the Nanopore reads and polished with Pilon v1.23 using the Illumina reads mapped with BWA v0.7.17-r1188. The sequences were annotated using Prokka v1.13. The results for APEC MS1170 showed a MDR phenotype, with resistance to amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftriaxone, gentamicin, streptomycin, sulfisoxazole and tetracycline. The complete genome sequence of MS1170 comprised 4,865,878 bp, which included a single contig chromosome of 4,735,839 bp along with three plasmids of 122,112 bp, 6,375 bp, and 1,152 bp, respectively. Multilocus sequence typing (MLST) analysis showed that strain MS1170 belonged to ST127, pathogenic phylogroup B2. A large number of acquired antimicrobial resistance genes were identified by ResFinder 3.2, including genes encoding resistance to aminoglycosides [aac(3)-Vla], β-lactams (blaTEM-1), folate pathway antagonist (sul1), and an unusual MDR gene mdf(A) that is responsible for fluoroquinolone, macrolide, phenicol, rifampicin, and tetracycline resistance. The virulence genes found in this strain share similar contents with extraintestinal *E. coli* (ExPEC), thus allowing for a potential zoonotic risk that may also cause human disease. The genome sequence of APEC MS 1170 provides valuable information on the resistance mechanisms and virulence characteristics of an avian pathogenic *E. coli*, as well as information for tracing the potential spread of this MDR clone from poultry product to the consumers.

**Key Words:** avian pathogenic *Escherichia coli*, multidrug-resistant, whole-genome sequencing, zoonotic, broiler

### 249 A Microbial Diversity Survey of US Broilers.

Morouj Al-Ajeeli*, Terry Lester, Brian Lee, Takashi Hamasaki, Walter Blalock, Tia O’Brien, Tomohiro Hamaoka, Calpis America, Peachtree City, Georgia, United States.

Poultry is a common source of foodborne illness, and pathogenic challenges facing these birds are of great concern to the broiler industry. Chicken gut microbiota plays a critical role in overall health and performance, so understanding the diversity of the bacterial community is essential for improving the poultry G1 tract microbiome. The objective of this survey was to isolate certain bacteria in fresh broiler fecal samples collected from commercial houses in eight states. A total of 866 fecal samples were collected from commercial broiler farms between January 2015 and December 2019. These fresh fecal samples were collected, immediately transported on ice, and then analyzed within 24 hours after the sampling. Six different media were selected initially for detection and were prepared by following the modified Mitsuoka method (Mitsuoka, 1971) based on the microorganism species, genus, or family that was being targeted. These included *Lactobacillus*, total anaerobes, Enterobacteriacea, *Enterococcus*, *Clostridium perfringens*, and *Staphylococcus*. Bacterial enumeration data were analyzed using the procedure of SAS 9.4, and the statistical significance was obtained using Tukey-Kramer at P ≤ 0.05. The microbial survey revealed variations in microbial load, showing the ranges for *Lactobacillus* (8.75 to 9.00 log CFU/g), total anaerobes (9.06 to 9.28 log CFU/g), *Enterobacteriacea* (6.87 to 7.69 log CFU/g), *Enterococcus* (6.39 to 6.87 log CFU/g), *Clostridium perfringens* (2.60 to 4.09 log CFU/g), and *Staphylococcus* (6.70 - 7.45 log CFU/g). Samples were also examined from different seasons from 2015 to 2019. Bacterial levels were significantly different (P ≤ 0.05) between winter and summer season. *Enterobacteriacea* average levels were 6.64 CFU/g in December and 6.67 log CFU/g in January but were 7.24 log CFU/g and 7.62 log CFU/g in August and September, respectively. *Enterococcus* was measured at 6.11 log CFU/g in January versus 8.00 log CFU/g in June, while *Staphylococcus* was measured at 5.72 log CFU/g in January versus 7.01 log CFU/g in July. This leads to the conclusion that microbial loads vary not only between regions, but also between different times of the year. This survey establishes a better understanding of various
microorganisms that currently live in the gut microbiota and how environmental conditions affect microbial load.

**Key Words:** microbial survey, broiler, fecal samples, gut microbiota, bacteria

250 **Effects of conidial concentration and timing on quality index and deoxynivalenol accumulation in corn inoculated with Fusarium graminearum.** Cai M. Wu*, Guangmang Liu, Institute of Animal Nutrition, Sichuan Agricultural University, Chendu, Sichuan, China.

Deoxynivalenol (DON) is a common mycotoxin in corn that mainly produced by fungus *Fusarium graminearum*. The objective of this study was to investigate effects of conidial concentration and days of post-infection (dpi) on quality index (QI) and DON accumulation in corn inoculated with *Fusarium graminearum*. Corn were irradiated using 60Co-γ radiation and assigned into 3 treatments (inoculated with 1 × 10⁵, 1 × 10⁶ or 1 × 10⁷ conidia/g of *Fusarium graminearum*), with 3 replicates per treatment and 20 triangle flasks per each replicate. The study last 60 d and other culture conditions were shown as follows: corn moisture 22%, culture temperature 25±2°C, humidity 75% ± 5%, and oxygen flux 1020 m²/m³. The corn QI and DON accumulation were measured on 0, 5, 10, 15, 20, 30 and 60 dpi. Data were analyzed using SAS PROC MIXED model with treatment means separated via Tukey’s Multiple Range Test. Statistical significance was considered at *P*<0.05. Results showed corn quality was decreased gradually with the increase of time post-inoculation, but no significant difference (*P*>0.05) among treatments. From d 0 to 60, there was a quadratic curve (firstly decreased and then increased) effect (*P*<0.05) on amino acids concentrations with the increase of time post-inoculation; crude fat, starch, crude fiber, protein solubility and gross energy of corn samples were decreased linearly (*P*<0.05). However, the acid value showed a linear increased pattern (*P*<0.05). Both mold counting and concentration of DON had the quadratic curve (firstly increased and then decreased) variation (*P*<0.05). There was a dose-dependent relationship between DON concentration and *Fusarium graminearum* inoculation dose. The accumulation regular of DON was shown as follows: DON were detectable on 15 dpi, their concentration were 0.17, 0.22 and 0.23 mg/kg in 3 treatments, respectively; on 20 dpi, concentration of DON were highest, their concentration were 0.41, 0.54 and 1.14 mg/kg in 3 treatments, respectively; between 21 to 60 dpi, DON concentration of 3 treatments decreased gradually. In conclusion, *Fusarium graminearum* decreased corn quality within 60 dpi and mainly produced DON during 15-20 dpi. The result indicates that corn, as main feed material of poultry, should short storage time and avoid to be contaminated with *Fusarium graminearum*.

**Key Words:** corn, *Fusarium graminearum*, quality, deoxynivalenol,

251 **Effect of pH on PAA efficacy in reducing foodborne pathogens on chicken wings.** Sasikala Vaddu,* Jasmine Kataria, Estafania Novoa, Amanda Elisa Moller, Avani Gouru, Manpreet Singh, Harshavardhan Thippareddi, *Poultry Science, University of Georgia, Athens, Georgia, United States.*

Peroxy acetic acid (PAA) is widely being used as an antimicrobial in poultry processing, specifically in the chiller. While the natural pH of PAA solutions is between 4.5 and 6.0 at the concentrations used, poultry processors adjust the pH to ≥8.0 to maintain product yield. The objective of the study was to evaluate (i) efficacy of PAA different concentrations, pH, and contact times against *Salmonella, E. coli* and *Campylobacter* and (ii) use of *E. coli* as a surrogate for *Salmonella* and *Campylobacter* to conduct validations studies for poultry processing. Fresh chicken wings (0.45 Kg) were inoculated with a cocktail of nalidixic acid resistant *Salmonella Typhimurium* (STNR), rifampicin resistant *Escherichia coli* (EC⁵R, five strains) and gentamicin resistant *Campylobacter coli* (CC⁵R) followed by 15 min of attachment time. Inoculated chicken wings were immersed in PAA solutions of 50, 250 and 500 ppm adjusted to pH 8.2 and 10.0 as well as non-adjusted PAA solutions for 10 s and 60 min. Treated chicken wings were rinsed for 1 min in chilled BPW (100 mL) containing 0.1% sodium thiosulfate to neutralize residual effect of PAA. Serial dilutions were prepared (peptone water [PW] for *Campylobacter* enumeration; PW containing 100 ppm nalidixic acid or 100 ppm rifampicin to enumerate STNR and EC⁵R, respectively) and spread plated on Campy-cefx agar containing gentamicin (200 ppm; CCGA) or plated on Petrifilm APC for enumerating *Salmonella* and *E. coli* populations. CCGA plates were incubated at 42 ± 1°C for 48 h under microaerophilic atmosphere. Non-PAA treated inoculated wings were used as control. Three replications were conducted for each treatment and Fishers’s LSD was used to separate the means (α=0.05). Immersion of inoculated chicken wings in PAA at different concentrations reduced (*P* ≤ 0.05) *Salmonella, E. coli* and *Campylobacter* populations compared to the control. Adjusting pH to 8.2 and 10.0 did not affect (*P>*0.05) the PAA efficacy. Immersion of chicken wings in 500 ppm of PAA (non-pH-adjusted) for 60 min resulted in greater microbial reductions (*P* ≤ 0.05) of *Salmonella, E. coli* and *Campylobacter* populations of 2.56, 2.53 and 1.90 log₁₀ CFU/mL, respectively. Higher concentrations and longer exposure times resulted in greater reductions (*P* ≤ 0.05) of *Salmonella, E. coli* and *Campylobacter* populations and increasing pH of PAA solution did not affect (*P>*0.05) its efficacy. Good correlation (r=0.93) was observed between *E. coli* (surrogate) and *Salmonella* populations reductions suggesting that *E. coli* can be used as a surrogate for *Salmonella* to conduct validation studies in poultry processing.

**Key Words:** *Salmonella*, *Campylobacter*, *E. coli*, peroxyacetic acid, chicken wings

252 **Sodium butyrate as potential anti-inflammatory...**
compound to reduce *Salmonella Enteritidis* colonization in chickens. Anamika Gupta1, Mohit Bansal1, Abhinav Upadhyay2, Narayan Rath1, Rohana Liyanage3, Xiaolun Sun1, Annie M. Donoghue1, ‘Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, 1University of Connecticut, Storrs, Connecticut, United States, 3Chemistry & Biochemistry, University of Arkansas, Fayetteville, Arkansas, United States.

*Salmonella* Enteritidis (SE) is a major poultry associated food borne pathogen that causes enteric illnesses in humans. Short-chain fatty acids such as butyrate is a microbial metabolite known to modulate the inflammatory response. Therefore, the objective of the present study was to determine the effect of sodium butyrate (SB) on the colonization of SE in primary chicken enterocytes. Also, the effect of SB on the proteome of primary chicken enterocytes, challenged with SE was investigated. The primary chicken enterocytes culture model was developed by using day-old broiler chicks. A growth curve assay was conducted to determine the sub-inhibitory concentration (SIC) of SB (363, 181.5, 90.75, 45, 22, and 11 mM) against SE. The effect of sub-inhibitory concentrations (SICs) of SB on SE adhesion and invasion of primary chicken enterocytes was determined. The effect on mRNA expression of inflammatory genes (IL1β, Il8, Mmp9) was investigated using RT-qPCR. For cell culture assays and RT-qPCR gene expression data was analyzed by using One-way ANOVA in Graph-pad 7 Software. The tryptic peptides of treated and control cells were analyzed using tandem mass spectrometry and the differentially regulated proteins were evaluated using a Scaffold proteome software (P<0.05). For all assays, triplicate samples were used, and the experiment was repeated two times. The two SICs of SB against SE were 22 mM and 45 mM and reduced SE adhesion by ~1.7 Log CFU/mL and ~1.8 Log CFU/mL and invasion by ~2 Log CFU/mL and ~2.93 Log CFU/mL respectively in primary chicken enterocytes (P<0.05). The expressions of inflammatory genes were significantly downregulated by SB as compared to control (P<0.05). Proteomic analysis revealed that SB significantly downregulates the expression of proteins in SE infected primary chicken enterocytes involved in host actin cytoskeleton rearrangements, pro-inflammatory innate immune response, and intracellular trafficking of SE in chicken’s intestinal tract. In contrast, SB treatment in SE infected cells upregulated (P<0.05) protein involved in bacterial killing during the pro-inflammatory signaling pathway. Results suggest the inhibitory potential of SB against SE colonization and inflammation in chickens.

**Key Words:** *Salmonella*, Primary chicken enterocytes, sodium butyrate, inflammation.

253 Effect of Windrow Composting on *Salmonella Enteritidis* Persistence in Poultry Litter. Aidan A. Talorico1, James Krehling1, Luis R. Munoz1, Kaicie S. Chasteen1, Amrit Pal1, Matthew Bailey1, Dianna Bourassa1, Kenneth Macklin1, ‘Poultry Science, Auburn University, Auburn, Alabama, United States, 2Poultry Science, Auburn University, Auburn, Alabama, United States.

One of the leading foodborne pathogens is nontyphoidal *Salmonella* spp.; poultry products are commonly associated with this pathogen. *Salmonella* can often be found in broiler litter, which can potentially allow for transmission between flocks. This study aims to track the persistence of *Salmonella* Enteritidis in broiler litter and to assess the effect of windrow composting on bacterial load. Commercially sourced chicks were randomly spread out over 16 pens (25 chicks/pen) containing fresh pine shavings. On day 6, every chick was administered a 1ml oral gavage of 1x107 cfu/ml of a nalidixic acid and novobiocin resistant strain of *Salmonella* Enteritidis. Chickens were fed a standard starter, grower, and finisher diet. Birds were terminated at 35 days of age, after which ceca were randomly removed from 5 birds/pen. Eight of the 16 pens were windrow composted. Windrow composting was performed by removing litter from pens and forming 2 separate 6m x 1m x 1m piles for 7 days without turning. *Salmonella* prevalence was monitored in each pen by 4 litter separate separate litter sampling techniques and in the bird by removing the ceca. Litter sampling techniques included litter grab, drag swab, boot covers, and roller swabs. Litter grab samples were collected for each pen, they were a composite sample of litter collected from beside the feeder, under the water line, and between the two. Litter grab samples were diluted and spot plated onto XLT4+ for enumeration. Swab samples were performed by covering roughly 75% of the surface area of the pens. Swabs and ceca samples were enriched in tetrathionate broth and streaked onto XLT4+. The initial sampling was performed on the day of flock termination and sampling took place biweekly (every 3-4 days) for a 2-week period. Data for litter counts were log transformed for statistical analysis and swab sampling methods were assessed based on the ratio of positive samples. Statistical differences were determined using two sample T test with significance determined when p <0.05. No differences were observed between control pens from litter grab counts or in any of the litter sampling methods. A significant reduction in microbial load (cfu/g) was observed after the windrow composting, although the ratio of positive swab samples remained the same. Microbial load dropped from 3.61 Log10(cfu/g) to 1.79 Log10(cfu/g) (P<0.01) after 7 days of composting when litter was placed back into pens. Mean microbial load of windrow pens was 3.54 Log10(cfu/g) vs 2.54 Log10(cfu/g) in control pens on the final sampling (P=0.02). The ration of *Salmonella* recovery from the ceca was 40% in all pens on the first day of litter sampling.

**Key Words:** *Salmonella*, litter sampling, composting, poultry litter, ceca

254 Growth of *Campylobacter* spp. and Carbon Dioxide Production in Primary Containers Incubated Anaerobically Growth of *Campylobacter* spp. and...
Carbon Dioxide Production in Primary Containers Incubated Anaerobically. Arthur Hinton*,1, Nelson Cox1, Arturo Levican1, 1Poultry Microbiological Safety and Processing Unit, U. S. National Poultry Research Center, Athens, Georgia, 1Pontificia Universidad Catolica de Valparaiso, Valparaiso, Chile.

The ability of Campylobacter to grow in primary containers incubated anaerobically was examined. Ten ml of media composed of (g/L) beef extract, 50; tryptose, 10; soluble starch, 10; sodium bicarbonate, 5.0; sodium lactate, 3.0; agar, 0.5 was added to 25 ml, 12.5 cm² culture flasks. The media was inoculated with 10⁴ cfu/ml of Campylobacter coli, Campylobacter fetus, Campylobacter jejuni, or Campylobacter lari; and the flasks were closed with plug-sealed caps, vented caps, or vented caps covered with Parafilm laboratory film. The flasks were incubated at 37°C for 48 h in an anaerobic atmosphere composed of 5% carbon dioxide, 10% hydrogen, and 85% nitrogen. After incubation, the number of cfu/ml of Campylobacter in the media was enumerated on a Campylobacter selective agar composed of Blood Agar Base #2 supplemented with 7.0% horse blood and the Blaser-Wang antibiotic mixture. The inoculated plates were incubated microaerobically at 37°C for 48 h, and cfu were counted. Additionally, the concentration of CO₂ in the flasks was measured in parts per million (ppm) with a CO₂ Sampling Data Logger. Significant differences in data were determined using GraphPad InStat statistical software. Results indicated that there was a 3-4 log increase in the number of Campylobacter recovered from media incubated in the plug-sealed flasks or the parafilm-sealed flasks, while there was a 1-2 log increase in the number of Campylobacter recovered from the vented flasks. Campylobacter growth in the plug-sealed and parafilm-sealed flasks was significantly greater than in the vented flasks. Furthermore, there was no significant difference in the growth of different species of Campylobacter when cultured in flasks with the same type of closure. The concentration of CO₂ in the inoculated flasks ranged from 1,000-4,000 ppm in the vented flasks; 9,000-14,000 ppm in the parafilm flasks; and 22,000-30,000 ppm in plugged flasks. The CO₂ concentration of the all vented flasks was significantly lower than the CO₂ concentration in the plugged flasks. While the CO₂ concentration of the parafilm flasks inoculated with C. coli or C. lari was also significantly lower than the CO₂ concentration of the plugged flasks, the CO₂ concentration of the parafilm sealed flasks inoculated with C. fetus or C. jejuni was not significantly different from the vented or plugged flasks. Conclusions indicate that the ability of Campylobacter to grow in the primary containers was related to the ability of the containers to retain CO₂ produced by the media and the bacteria. Utilization of this medium in closed containers will allow laboratories to simplify procedures for culturing Campylobacter.

Key Words: Campylobacter, carbon dioxide, anaerobic atmosphere, culture flask, growth

255 Development of probe-based multiplex real-time PCR assays for the rapid and accurate detection of avian pathogenic Escherichia coli. Reshma Ramachandran*,4, Chuan-Yu Hsu1, Li Zhang1, 1Poultry Science, Mississippi State University, Starkville, Mississippi, United States, 2Institute for Genomics, Biocomputing and Biotechnology, Mississippi State University, Starkville, Mississippi, United States.

Avian pathogenic Escherichia coli (APEC) known to cause colibacillosis and other related diseases in poultry causes significant economic losses to the poultry industry. Currently, due to the diverse genetic characteristics of APEC strains, there are no reliable rapid diagnostic tests for APEC surveillance or prevalence. Therefore, the objective of this study was to develop probe-based multiplex real-time PCR assays for the rapid and accurate detection of APEC. Two triplex TaqMan-real-time PCR assays were developed to target ybbw gene for E. coli confirmation and five major virulence genes (hlyF, ompT, iss, iroN, iutA) for APEC detection. TaqMan primers and probes were designed to target ybbw/hlyF/ompT genes synthesized with ABY, FAM and VIC dyes with QSY quencher, and iss/iroN/iutA genes synthesized with JUN, FAM, VIC dyes with QSY quencher within each triplex real-time PCR. These target genes were cloned into pGEM-T Easy vector and were transformed into E. coli NovaBlue competent cells. After extraction, the plasmids were verified for the correct insert by sequencing and were quantified by NanoDrop. The purified plasmids served as positive control to construct the standard curve and to test the amplification efficiency. No cross reactivity was observed between the different genes within each triplex TaqMan real-time PCR assays. Further, the amplification efficiencies and the coefficient of determination (R²) values will be evaluated and an efficiency between 90 and 110% will be considered acceptable. In conclusion, this multiplex real-time PCR strategy has the potential to be developed as a rapid and accurate diagnostic tool for the detection of APEC isolates and to differentiate them from commensal E. coli in poultry production.

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

256 Broiler house particulate matter, aerobiome and antibiotic resistant E. coli under “raised without antibiotics” production. Gregory Zock*,1, Lilong Chai1, Adelumola Oladeinde2,3, Samuel Aggrey1, Jasmine Johnson1, Yangyang Guo1, 1Poultry Science, University of Georgia, Athens, Georgia, United States, 2U.S. National Poultry Research Center, USDA, Athens, Georgia, United States.

Broiler houses introduce high levels of particulate matter (PM) and airborne bacteria which may lead to health concerns and pathogenic transfer in birds and handlers. The general shift towards antibiotic (AB)-free live broiler production indicates the need for active research on AB
resistant commensal bacteria that may persist in broiler houses under “Raised Without Antibiotic (RWA)” production. While studies have shown compositional profiles of PM and bacteria in broiler houses, there is no data on temporal or spatial changes in broiler house aerobiome or AB resistant bacteria. The purpose of this study was to determine the relationship between PM, litter factors (moisture and E. coli abundance), aerobiome, and AB resistant E. coli. About 125 Cobb-500 broiler chicks were raised in floor pens on “reused” litter for 49 days following RWA production conditions. A DustTrak DRX (TSI Inc) was used to measure airborne PM sizes of 1, 2.5, 4, and 10 micrometers. Open petri dishes with ECC CHROMagar™ or Brain Heart Infusion agar were placed in the broiler house to quantify levels of airborne coliforms, E. coli, and total bacteria. Bacterial and PM sampling took place at heights representing bird level (height 1), human level (height 2) and upper room circulation level (height 3) for 12 minutes. PM sizes consisted of 18-25% PM2.5 and 33-50% PM10 regardless of height or day of sampling. PM changed consistently throughout the study, and there was no difference between heights (P>0.05). No airborne E. coli was detected at day 0, however, E. coli was measured on day 7 at 2.98 and 3.76 log CFU/m³ for height 1 and 3. Airborne E. coli increased and ranged from 4.49-4.95 log CFU/m³ from day 14-28 and there was no difference between heights (P>0.05). In contrast, coliform bacteria concentration decreased at all heights from day 14-28. AB susceptibility testing was done on a selected number of E. coli isolates (n=71) with 66% of isolates susceptible to 14 antibiotics tested and 23% displaying multidrug resistance to two or more classes of antibiotics. A multiple regression test revealed that airborne E. coli was positively correlated to litter E. coli and PM2.5 (P<0.001) and negatively correlated to PM10 (P<0.001) with litter moisture having no effect. There was no correlation found between these physiochemical factors and concentrations of airborne coliforms.

Key Words: Broiler production, antibiotic-free, housing environment, dust, airborne bacteria

257 New Avian Pathogenic Escherichia coli O Serogroup Epidemiology for Poultry Health in Georgia. Nicolle Barbieri1*, Caleb Austin1, Robert Pasko1, Aline L. Oliveira1, Lisa Nolan1, Catherine M. Logue1, 1Population Health, University of Georgia, Athens, Georgia, United States, 2Infectious Diseases, University of Georgia, Athens, Georgia, United States, 3Poultry Science, University of Georgia, Athens, Georgia, United States.

Colibacillosis caused by Avian Pathogenic E. coli (APEC) is a costly disease for the poultry industry worldwide, significantly affecting one of the world’s cheapest sources of high-quality protein, resulting in mortality at the farm and carcass condemnation at slaughter. For years, geographic variation in APEC distribution has been known, with the most common E. coli serogroups representing >90% of characterized APEC which includes serogroups O1, O2, and O78. Currently, commercial vaccines are available specifically targeting these serogroups. Here, we propose to fill the gap in knowledge of APEC and perform an epidemiological study to investigate emerging O serogroups that may be contributing to poultry disease in Georgia. Our research group has developed a PCR-based-assay that identifies the 13 most frequently found O serogroups (including O1, O2, O8, O18, O24, O25, O44, O78, O86, O88, O103, O117 O119, and O186). We found that 72.2% of isolates from diagnostic APEC, and 25.3 % of the fecal isolates were positive for one of these 13 serogroups tested. The most prevalent serogroups identified among strains of APEC implicated in disease in Georgia include O78, O24, O2, and O25 with a prevalence of 22.8, 14.3, 10, and 8.6% respectively. While serogroups O8, O44, O18, and O186 (5.8, 5.6, 4.5, and 3.0% prevalence) were most common among fecal isolates collected. Epidemiological analysis of our data has allowed us to identify new emerging clones, such as O25 and O24 that were not previously associated with APEC before, and defining traits of the environment of the host that can potentially lead to E. coli-associated disease in production broilers in Georgia. Interestingly we found O8 and O18 serogroups in fecal chicken isolates that are common serogroups found in E. coli associated with human urinary tract disease. With better knowledge of the populations of microbiota present, we can better predict vulnerable flocks and identify strategies for colibacillosis control at the farm level. Having a better knowledge of the current APEC causing disease we can identify new potential targets for vaccine development which could potentially reduce losses for producers in Georgia and elsewhere.

Key Words: APEC, O Serogroup, Colibacillosis

258 Rapid Quantification Validation and Confirmation of Salmonella for Comminuted Turkey. Savannah Forgey1*, Tyler Stephens2, Marcos Sanchez2, April Englishbey2, 1Animal and Food Science, Texas Tech University, Lubbock, Texas, United States, 2Hygiena, Camarillo, California, United States.

Introduction: Most Probable Number (MPN) is the United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) reference method for enumerating Salmonella with results taking up to 5 days. Currently, the poultry industry is searching for a rapid and reliable validated method to estimate Salmonella levels in comminuted turkey. Objective: The objective of this study was to validate BAX® System SalQuant (SalQuant) for comminuted turkey compared to the MLG MPN reference method. Materials and Methods: Comminuted turkey samples (N = 20) were inoculated on a 400 g basis with five samples per inoculation level: low (0.0 to 1.0 Log10 CFU/g), medium (1.0 to 2.0 Log10 CFU/g), high (2.0 to 3.0 Log10 CFU/g) and control (non-inoculated). Samples were stored at 4 - 8°C for 48 - 72 h for cold stressing. After the stress period, 325 g of the inoculated comminuted turkey samples were combined with 1,625 mL of BPW to create a primary enrichment. 30 mL of primary enrichment homogenate was
removed and combined in a sterile filtered Whirl-Pak bag with prewarmed 42°C BAX MP Media with antibiotics as a secondary SalQuant enrichment. The primary enrichment samples were incubated at 35°C for 18-24 h and analyzed using the BAX® System Real-Time Assay for Salmonella (RT Salmonella) for prevalence. SalQuant secondary enrichment samples were incubated at 42°C for 8 hours prior to analysis with RT Salmonella. The MLG MPN was performed following the MLG Appendix 2.05 through confirmation. Comparison using the BAX® System as a confirmatory method versus conventional plating requirements outlined in the MLG were evaluated. Estimated Salmonella levels from SalQuant, MPN with BAX® confirmation, and MLG MPN were compared at each inoculation level using JMP® v.14.3.0 with statistical significance at \( P \leq 0.05 \). Results: There were no statistical differences at any inoculation level between SalQuant and MLG MPN (\( P = 0.89 \)). SalQuant estimated the low, medium, and high inoculation levels at 0.55, 1.68, and 2.33 \( \log_{10} \) CFU/g, respectively, with 0.31 \( \log_{10} \) CFU/g standard error (SE). MLG MPN estimated inoculation levels at -0.04, 1.57, and 2.49 \( \log_{10} \) CFU/g, respectively, with upwards of 0.64 \( \log_{10} \) CFU/g SE. Both MPN confirmation methods, BAX® System and MLG plating, were 100% comparable.

Conclusion: SalQuant provides rapid, accurate, and reliable estimation of Salmonella with same-day results for comminuted turkey, whereas MLG MPN can take 24-26 h if confirming with the BAX® System, or up to 5 days for conventional plating confirmation.

Key Words: BAX® System SalQuant, Quantification, Estimation, Most Probable Number (MPN)

259 Equivalency of peracetic acid to chlorine as a shell egg surface sanitizer. Deana R. Jones*,†, Javier S. Garcia*, Richard K. Gast†, Garrett E. Ward‡, †US National Poultry Research Center, Egg Safety and Quality Research Unit, USDA Agricultural Research Service, Athens, Georgia, United States, ‡Poultry Science, University of Georgia, Athens, Georgia, United States.

In the US, all shell eggs processed under the USDA Agricultural Marketing Service voluntary grading standards must receive a sanitizing rinse after leaving the washing process of 100 – 200 ppm chlorine or its equivalent. A study was conducted to determine the concentration of peracetic acid (PAA) which would be equivalent to 100 – 200 ppm chlorine under the required washing conditions for shell eggs. Three isolates of Salmonella spp. (Enteritidis, Braenderup, and Typhimurium), as well as Enterobacter cloacae (indicator organism frequently detected in shell egg processing environment), were utilized as inocula. Sanitizing treatments were: negative control; deionized water; 100 and 200 ppm chlorine; and 50 – 500 ppm peracetic acid (7 concentrations). Over three replications, up to 30 intact eggs were assessed for each inoculum and sanitizing treatment combination. Organism enumeration data was analyzed by ANOVA with a general linear model and least square means separation. Prevalence of viable, but not culturable, organisms was analyzed by Chi-square. Considering all microbial numbers, 100 and 200 ppm chlorine had 2.6 and 2.3 \( \log \) cfu/ml remaining on shell surface; 50 and 100 ppm peracetic acid had 1.9 and 1.0 \( \log \) cfu/ml remaining, respectively, compared to untreated control average of 3.8 \( \log \) cfu/ml (~0.001). Salmonella Typhimurium was least resistant to shell sanitizer treatments. Peracetic acid concentrations > 250 ppm did not produce significant reductions in microbial populations as PAA concentration increased. Culturing for the prevalence of viable and injured organisms, 400 – 500 ppm PAA resulted in fewer eggs (\( P < 0.0001 \)) being positive for Salmonella spp. Enterobacter cloacae was culturable via enrichment from 99.4% of inoculated eggs, regardless of sanitizer treatment. The results of this study indicate that 50 -100 ppm PAA is equivalent to 100 – 200 ppm chlorine in reducing egg shell microorganisms. The use 400 – 500 ppm PAA resulted in a lower incidence of viable, but not culturable, Salmonella spp. on the shell surface. Enterobacter cloacae resulted in almost 100% viable, but not culturable, organism recovery for all sanitizing treatments and should be considered as an indicator organism when studying processing facility sanitation procedures.

Key Words: shell egg, surface, sanitizer, peracetic acid, chlorine

260 Evaluating the efficacy of Peracetic acid on Salmonella and Campylobacter on chicken wings at various pH levels. Jasmine Kataria*†, Sasikala Vaddu†, Estafania Novoa†, Gaganpreet Sidhu†, Harshavardhan Thippareddi†, Manpreet Singh†, †Poultry Science, University of Georgia, Athens, Georgia, United States, ‡Poultry Science, University of Georgia, Athens, Georgia, United States.

Peracetic acid (PAA) is commonly used as an antimicrobial during poultry processing to reduce the pathogen load on poultry and poultry products. However, limited research is available on the effects of higher pH on the efficacy of PAA against Salmonella and Campylobacter. Therefore, the objective of this study was to determine the efficacy of PAA in reducing Salmonella and Campylobacter populations on chicken wings adjusted to various pH levels. Chicken wings (0.454 kg each i.e. ~6-7 wings/treatment) were spray-inoculated with nalidixic acid-resistant (200 ppm) Salmonella Typhimurium (~7 \( \log_{10} \) CFU/mL) and gentamicin-resistant (200 ppm) Campylobacter coli (~6-7 \( \log_{10} \) CFU/mL) and allowed an attachment period of 15 min. Inoculated wings were treated with PAA by immersion for 10 s or 60 min at 4 - 6 °C. The treatments included 50 ppm (0.005%) and 500 ppm (0.05%) PAA at three pH levels (8.2, 10, and 11) or sodium hydroxide (NaOH, pH 11). Surviving populations of Salmonella and Campylobacter were determined by rinsing the chicken wings post-treatment in Buffered Peptone Water (100 mL) for 60 s. Serially diluted rinsates were plated on Aerobic Plate Count Petrifilm™ and Campy-cefex agar for Salmonella and Campylobacter.
enrollment, respectively. Statistical differences between the treatments were determined using ANOVA with Least Square Means at p ≤ 0.05. Salmonella levels were reduced (p ≤ 0.05) by about 1 and 2 logs when chicken wings were immersed in 500 ppm PAA for 10 s and 60 min, respectively. Higher pH did not improve the antimicrobial efficacy of PAA (50 or 500 ppm) against Salmonella on chicken wings. Campylobacter reductions of about 2.5 logs were observed on chicken wings immersed in PAA (50 or 500 ppm) for 10 s or 60 min as compared to NaOH treatment at pH 11 (p ≤ 0.05). Moreover, immersion time and the higher pH of antimicrobial solutions did not affect (p > 0.05) the antimicrobial efficacy of PAA (50 or 500 ppm) against Campylobacter. The results from this study demonstrated that higher pH did not have an impact on the antimicrobial efficacy of PAA and further suggest effective contact time and concentration combinations of PAA for use in poultry processing.

Key Words: Salmonella, Campylobacter, Peracetic acid, pH

261 The antibiotics resistant and biofilm forming Salmonella species isolated from poultry of tribal areas in Central India. Smita Bordoloi1,2, Anup Kollanoor Johny3, Shivani Basu4, Dipak K. Nair5, Divakar V. T. Nair6, NDVSU, Bhopal, Madhya Pradesh, India.

Poultry are the important reservoir of many zoonotically important pathogens, of which Salmonella is of prime importance. Biofilm facilitates the adherence of microorganisms to surfaces and protect them from host immune system. Madhya Pradesh occupies an important place in tribal population in agroclimatic zone V (central Narmada valley) in Central India. The multidrug resistant salmonella strains can cause serious food born zoonotic infections. The present experiment was conducted to evaluate the antimicrobial sensitivity and multidrug-resistance for Salmonella species isolated from poultry of tribal areas of Madhya Pradesh from Hoshangabad and Narsingpur district. A total of 721 samples (cloacal swab, dry faeces, feed, water and egg shell) from poultry consisting of broilers (297), layers (316) and breeders (108) in rainy, winter and summer season, were collected. For phenotypic isolation of Salmonella organism, samples were inoculated in pre enrichment broth, followed by enrichment in Rappaport Vassilikos broth than selective plating on MacConkey agar, brilliant green agar and Xylose Lysine Deoxycholate agar. A total of 64 isolates were phenotypically characterized by motility, oxidase, catalase, indole, methyl red, Voges Proskauer, triple sugar iron agar, urease and later confirmed by latex agglutination test. Biochemically they were detected as Salmonella species and further they were confirmed by latex agglutination test (100%). The overall incidence was 8.87%. While for broilers, layers and breeders incidence of salmonellosis was 9.09%, 8.54% and 9.20%, respectively. Genotypically these isolates were confirmed and characterized using polymerase chain reaction for four genes 16S rRNA (100%), invA (100%), Salmonella enterica ser. Typhimurium (70.31%) and Salmonella enterica ser. Gallinarum-Pullorum (0%). Salmonella enterica ser. Typhimurium positive isolates were strongly biofilm producers in broiler, layer and breeder (62.50%, 62.50% and 66.66%). In antibiogram most bacteria were showing resistant for norfloxacin (81.48%), ciprofloxacin ampicillin/sulbactum in broiler, layer and breeder respectively and were sensitive for Vancomycin. In antibiogram bacteria were resistant for antibiotics norfloxacin (81.48%), ciprofloxacin (92.59%) and ampicillin/sulbactum (80%) and sensitive for Vancomycin. The history showed that these antibiotics were never used in these farms therapeutically or as a preventive measures still showing resistant in this study; there may be probability of resistant gene transfer through Salmonella from other species, which can be revealed in further study. The salmonellosis can be eliminated by following strict hygienic practices.

Key Words: multidrug-resistance, Biofilm, Salmonella, layer, tribal

262 Effect of Lactococcus lactis Cell-Free Extract and Bacitracin against Multidrug-Resistant Salmonella Heidelberg. Brigitta P. Yaputri1,2, Divek V. T. Nair2, 1Food Science & Nutrition, University of Minnesota Twin Cities, Minneapolis, Minnesota, United States, 2Animal Sciences, University of Minnesota Twin Cities, St. Paul, Minnesota, United States.

Multidrug-resistant Salmonella Heidelberg (MDR SH) is a significant foodborne pathogen causing outbreaks in the United States, through contaminated poultry meat and products. SH strains isolated from the past outbreaks have been found resistant to multiple antibiotics, including bacitracin. Bacitracin is a polypeptide antibiotic known for its activity against Gram-positive bacteria, such as Clostridium perfringens, that causes necrotic enteritisid in poultry. In our previous studies, we found that Lactococcus lactis, a Gram-positive beneficial bacterium of dairy origin, can inhibit SH in vitro. The objective of the current study was to determine the efficacy of L. lactis cell-free extract (CFE - bacteria-free filtrate obtained from centrifugation and filtration of growing L. lactis culture), in increasing the sensitivity of MDR SH towards bacitracin. The experiments were conducted in 12-well tissue culture plates following a completely randomized design with a 6X3X2 factorial treatment structure. Each well contained 2 ml TSB added with 0, 5, 7.5, 10, 12.5 or 15% (v/v) of L. lactis CFE. Three different concentrations of bacitracin tested were 128, 256, and 384μg/ml. The treatments (bacitracin and CFE) were tested alone or in combination to determine their effect on MDR SH growth (2011 ground turkey outbreak isolate inoculated at ~5 log10 CFU/ml/well). Since the addition of CFEs reduced the pH of the test medium, pH controls were set for each concentration in addition to the negative and positive controls. Plates containing the treatments were incubated at 37°C for 24 h. A microplate reader was used to measure the optical density absorbance values (OD600) to
determine the growth of MDR SH at 0h and 24h of incubation. Six samples were included for each treatment, and the experiment was repeated 6 times. Data were analyzed using ANOVA (P<0.05 considered significant). The CFE resulted in a concentration-dependent reduction of MDR SH when functioned alone against the pathogen (P<0.05), indicating the direct antimicrobial property of the CFE. The CFE concentrations ≥12.5% resulted in a synergistic activity with all the three tested concentrations of bacitracin and resulted in a maximum reduction of MDR SH (P<0.05). The results revealed that the combination of CFE (potentially spray-dried) and bacitracin could be a highly effective intervention through the feed to control Gram-negative pathogens such as S. Heidelberg in poultry. However, further testing in poultry towards this effect is warranted (Funded by University of Minnesota Undergraduate Research Opportunities Program and Minnesota Agricultural Experimentation Station Grant# MIN-16-120).

**Key Words:** Lactococcus lactis, cell free extract, bacitracin, Salmonella Heidelberg, multidrug-resistant

### 263 Understanding possible contributing factors of embryonic mortality

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Eggs produced by broiler breeders are an essential component to the broiler industry as they generate the next generation of broilers. However, little is understood about various factors that contribute to embryonic mortality. Specifically, it is unclear if bacterial exposure during development and location of bacterial colonization contribute to embryonic mortality. Therefore, to determine if bacterial exposure and/or bacterial location are present with embryonic mortality, eggs were collected twice daily from hens exposed to rooster (8 hens: 1 rooster) starting at week 37 through week 47 (hen age) from 24 different pens. Pen number and date were recorded on the egg. Eggs were set at the conclusion of the week (288 eggs in a Sportsman 1502). At embryonic days 7, 14, and 21 eggs were checked for normal embryonic development. At days 7 and 14, eggs were candled using a handheld LED flashlight. Eggs that were found to have ceased embryonic development due to delayed growth were pulled from the incubator. Additionally, normally developing eggs (NDE; n=4) at each time point were pulled to determine normal bacterial colonization. At day 21, eggs that failed to hatch or pip were considered to have delayed development. All eggs collected were transported to the lab; aseptically cleaned with 70% ethanol; embryonic age determined; swabbed with sterile cotton swabs along the outer membrane of the egg shell near the air sac and the bottom end of the egg; plated on MacConkey and Sheep Blood Agar; incubated at 37°C; and bacterial counts recorded the following day. Data were analyzed using linear modeling in SAS. Of the 555 eggs sampled, 9% yielded at least one colony. Average bacterial counts increased 15,350 and 8,378% in the air sac and bottom of the eggs, respectively, compared to NDE (P < 0.0001). No difference was detected when comparing sampling location in either NDE (-0.02% air sac vs bottom) or embryonic mortality (12%, air sac vs bottom; p>0.8). While this data aggregates the bacterial types, this sharp contrast in number of bacterial colonies and numerical change in the location of bacterial colonization may be associated with increased the embryonic mortality but additional data is needed to better understand bacterial colonization of eggs and its contribution to embryonic mortality.

**Key Words:** air sac, bacterial colonization, broiler breeders

### 264 Salmonella and Campylobacter influence the microbiota response of skin-on, bone-in chicken thighs treated with different antimicrobials

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For antimicrobials to be utilized properly in poultry processing, it is important to understand the effect these antimicrobials have on key pathogens and the subsequent microbiota of poultry carcasses. Therefore, the objective of this study was to determine the influence of two antimicrobials, peracetic acid (PAA) and acidified sodium chlorite (ASC), on the microbiota of chicken thighs inoculated with Salmonella and Campylobacter. In two independent trials, chicken thighs (N=360, n=10, k=9, 2 time points, 2 inocula) were inoculated with either a cocktail of S. Enteritidis, Heidelberg, Infantis, Kentucky, Typhimurium or a cocktail containing C. jejuni (10⁷ and 10⁸ CFU/mL) and incubated at 4 ºC for 90 m. Inoculated thighs were independently dipped for 10 s into sterile bags containing 400 mL of the following treatments: Tap water (TW), 800 or 1100 ppm of ASC (pH 2.4 and 2.8), and 200, 350, 500, or 650 ppm of PAA. After treatment, samples were immediately stored at 4 ºC. At 0 and 24 h, thighs were rinsed in 150 mL of nBPW and subsequent rinsates were aliquoted for pathogen detection (n=10) and microbiota analyses (n=5). Salmonella and Campylobacter were enumerated using the drop plated method. Genomic DNA of rinsates was extracted, and the 16S rRNA was sequenced (Illumina MiSeq platform). Pathogen data were analyzed using 2-Way ANOVA in JMP 14, with means separated by Tukey’s Protected HSD (P<0.05). Microbiota data was filtered and aligned using the QIIME 2-2019.7 pipeline, with data considered significant at P ≤ 0.05 for main effects and Q<0.05 for pairwise differences. The results of the study demonstrated that both 800 ppm ASC (pH 2.4) and 650 ppm PAA were effective at reducing inoculated Salmonella and Campylobacter (P<0.05). In addition, the inocula (Salmonella or Campylobacter) and trial had a major influence on Alpha and Beta diversity and compositional changes of the microbiota (P<0.05, Q<0.05). Because the inocula species and trial had significant effects
on the diversity analyses, the analysis of compositional differences of microbiomes (ANCOM) was separated by inocula and trial on the species level. In the Campylobacter study, Campylobacter and Bacillus spp. were significantly different among treatments and time, regardless of trial (W=432, 430). During Trial 2 of that study, there was a significant relative abundance of Pseudomonas and Proteus spp. (W=432, 432). In the Salmonella study, Enterobacteriaceae and Bacillus spp. were significantly different in treatments across time, regardless of trial (W=432, 430). Overall, 800 ppm ASC (2.4 pH) and 650 ppm PAA were the most effective at altering the microbiota composition of chicken thighs when inoculated with Salmonella or Campylobacter.

**Key Words:** broiler thighs, peracetic acid, acidified sodium chlorite, 16S rRNA, pathogens

265 **Application of Amplon® in Combination with Peroxyacetic acid for the Reduction of Salmonella Typhimurium and S. Reading on Skin-on, Bone-in Tom Turkey Drumsticks.** Elena G. Olson†, Lindsey A. Wythe, Dana K. Dittoe, Kristina M. Feye, Steven Ricke, Food Science, University of Arkansas, Fayetteville, Arkansas, United States.

Peroxyacetic acid (PAA) has become an important component of pathogen reduction in poultry processing, but there are potential concerns for continued exposure. The objective was to evaluate the effects of PAA and Amplon® (AMP) used alone or in the combination. Bone-in tom turkey drumsticks (N=100, n=10, k=5, 0 and 24 h) per study were obtained and inoculated with either Salmonella Typhimurium or S. Reading. The inocula were allowed to adhere to the drums at 4 °C for 60 min for a final attachment of 10^5 and 10^6 CFU/g per S. Typhimurium and for S. Reading. Drumsticks were treated with a no treatment control (NTC); tap water (TW); TW+500 ppm PAA (PAA); TW+500 ppm AMP, pH 1.3 (AMP); TW+PAA+AMP (PAA+AMP). Treatments were applied as short duration dips (30 s) and allowed to drip for 2 min. Following treatment, drums were stored at 4 °C until microbial analyses at 0 and 24 h. Drums were rinsed in nBPW and spot plated for total aerobes and Salmonella. Bacterial counts were Log_{10} transformed and analyzed using n-way ANOVA. All treatments reduced S. Reading on turkey legs at both 0 and 24 h (P<0.0001; P>0.0001). At 24 h, drums treated with PAA+AMP (3.92 Log_{10} CFU/g) had less S. Reading than NTC, TW, and AMP. Treatment by time interactions were observed among drums treated with S. Typhimurium and total aerobes during both studies (P<0.0001, P=0.02, P<0.0001). During the S. Reading and Typhimurium study, all treatments reduced Salmonella and total aerobes on drums. During the S. Typhimurium study, drums treated with PAA+AMP had the lowest load of S. Typhimurium and total aerobes (3.87 and 6.27 Log_{10} CFU/g). The combination of AMP+PAA may exhibit a synergistic effect in reducing Salmonella on turkey drums, thus increasing the safety of turkey products for consumers.

**Key Words:** turkey, peroxyacetic acid, Amplon®; pathogen reduction, Salmonella

266 **In ovo Feeding of Clostridiale Strains Modulate Gut Microbiome.** Shaymaa M. Abousaad*, Ramon Malheiros1, Peter R. Ferket1, ‘Prestige Department of Poultry Science, North Carolina Satate University, Raleigh, North Carolina, United States, *Biology, North Carolina A&T State University, Raleigh, North Carolina, United States.

The use of live microorganisms to manage enteric health is becoming a feasible practice, especially when administered early in a broiler’s productive life. Strains within the Clostridiale order, including many butyric acid-producing anaerobes, are found in the intestines of healthy animals have been shown to promote growth performance and improve gut health. A novel mixture of unique Clostridiale strains, which may have beneficial effects on the microbiome development of perinatal poultry, was constructed. The objective of this study was to examine the effect of this mixture delivered by in ovo feeding (IOF) on the cecal microbiome and volatile fatty acid profile. Two experiments were conducted to measure the effect on gut microbiome of broiler chickens. Viable eggs of equal weight (± 0.5 std.) were segregated for different experimental treatments. At ~17 day of incubation, 100 µL of either 10^1, 10^3, 10^5 or 10^7 CFU/mL was administrated into the embryonic amnion. In both studies, a saline control was also injected, and a sham control (i.e., no injection) was used in one of the studies. Cecal material from 21 d old chicks were obtained and microbiome metabolites were subjected to analysis using GC for quantification of acetate, lactate, and butyrate. Analysis for the microbiome profiles showed significant difference for both 10^5 CFU/mL and 10^7 CFU/mL from the saline control. The 10^5 CFU/mL dosage had greater proliferation of the Clostridiale strains in the cecum. Butyrate was detected in samples analyzed for the 10^5 CFU/mL dosage with average concentration of 0.14 mg/g cecum material. These data suggest that the Clostridiale mixture influences the microbiome and volatile fatty acid profile of the cecum in broiler chickens. In conclusion, in ovo administration of 10^5 CFU/mL per egg into the amnion of the chicken embryos can modulate the microbiome that may maintain gut health.

**Key Words:** in ovo feeding, Clostridium, microbiome, cecal volatile fatty acids, broilers

267 **Application of cetylpyridinium chloride (CPC) on Poultry Skin for the Reduction of Nalidixic Resistant Salmonella Typhimurium and S. Infantis on Chicken Skin.** Elena G. Olson†, Lindsey A. Wythe, Dana K. Dittoe, Kristina M. Feye, Steven Ricke, Food Science, University of Arkansas, Fayetteville, Arkansas, United States.

Firmly attached bacteria are not eliminated with general rinsing methods. More so, poultry skin produces a buffering
effect which hinders the ability of sanitization agents to work effectively. Thus, the objective of this study was to evaluate the effects of two leading pre- and post-chill antimicrobials: peroxyacetic acid (PAA) and cetylpyridinium chloride (CPC). Chicken breast skins (4 x 4 cm; N = 100, n = 10, k = 5) per study were obtained and inoculated with either Salmonella Typhimurium or S. Infantis. The inocula were allowed to adhere to the skins at 4 °C for 30 min for a final attachment of $10^6$ CFU per gram of chicken for S. Typhimurium and S. Infantis. Skins were then shaken for 30 s to remove the excess fluid. Remained bacteria were considered firmly attached. Chicken skins were treated with a no inoculation - no treatment control (NINTC), no treatment control (NTC), tap water (TW), TW + 500 ppm PAA (PAA), or TW + 5000 ppm CPC (CPC). Treatments were applied as short duration dips (30 s) in 50 mL and the excess fluid was shaken off (30 s). Immediately after, skins were rinsed in nBPW, agitated via stomacher (1 min), and spot plated for Salmonella. Subsequent bacterial counts were $\log_{10}$ transformed. Data were analyzed using a One-way ANOVA in JMP 15 with means being separated by Tukey's HSD (P < 0.05). Results demonstrated that there was an effect of treatment on both species of Salmonella (S. Typhimurium, P < 0.0001; S. Infantis, P < 0.0001). PAA was the only treatment to reduce S. Typhimurium compared to NTC (P < 0.0001). Both PAA and CPC reduced S. Infantis on inoculated skins compared to those treated with NTC (P < 0.0001), but were not different from each other. In conclusion, PAA and CPC have different effects on chicken skin inoculated with different strains of Salmonella (Typhimurium vs Infantis).

**Key Words:** chicken skin, peroxyacetic acid, cetylpyridinium chloride, Salmonella short duration dip

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**268 The Effects of Cetylpyridinium Chloride for the Reduction of Salmonella Infantis on Skin-on, Bone-in Chicken Thighs.** Lindsey A. Wythe, Elena G. Olsen, Kristina M. Feye, Dana K. Dittoe, Steven Ricke, Food Science, University of Arkansas, Fayetteville, Arkansas, United States.

Salmonella, an increasingly problematic foodborne pathogen, can easily attach to poultry skin. As a consequence, validating uses of post-harvest antimicrobials are necessary to effectively reduce Salmonella spp. on skin-on poultry parts. Therefore, the objective of this study was to evaluate the effects of cetylpyridinium chloride, CPC, and peracetic acid, PAA, as short duration dips on skin-on chicken parts against a nalidixic acid (NA) resistant strain of Salmonella Infantis. Commercially obtained chicken thighs (N = 100, n = 10, k = 5, 2 time points) were inoculated with NA resistant S. Infantis inocula (1 mL per 25 g of chicken) and allowed to adhere for 60 min at 4 °C. An attachment of $10^6$ CFU per g of chicken (mean weight of 253.16 g) was obtained and confirmed via serial dilutions and subsequent spot plating on XLD+NA (64 µg/mL). The experimental treatments included: a no inoculum, no treatment control (NINTC); no treatment control (NTC); and 20 s antimicrobial dips in 150 mL of either tap water alone (TW); or with the addition of CPC (0.5%); or PAA (600 ppm). Immediately following treatment, thighs to be analyzed at 24 h were stored at 4 °C. At 0 and 24 h, thighs were rinsed in 150 mL of nBPW for 1 min and the subsequent rinsate was collected for downstream analysis. Rinsates were serially diluted, spot plated on XLD+NA (64 µg/mL) and TSA, and incubated aerobically at 37 °C for 24 h. Microbial counts were log-transformed and reported as Log$_{10}$ CFU of bacteria per gram chicken thigh (CFU/g). Data were analyzed using two-way ANOVA in JMP 15.0 with means separated using Tukey’s Protected HSD (P < 0.05). The results of the current study demonstrated that there was an interaction of treatment and time on the load of Salmonella and total aerobic bacteria on chicken thighs (P < 0.05). Thighs treated with CPC and PAA had the lowest load of Salmonella compared to all controls and were not different from each other. At 24 h, thighs treated with CPC and PAA had 3 and 2.5 Log$_{10}$ CFU/g less of Salmonella compared to the controls. At 0 h there were no differences among experimental treatments; however, at 24 h thighs treated with CPC and PAA were different than those treated with TW and NTC. In addition, thighs treated with CPC and PAA had 2.5 and 2 Log$_{10}$ CFU/g less of total aerobic bacteria compared to TW and NTC. At 24 h, total aerobic bacteria on CPC and NINTC treated thighs were not different, indicating that the treatment of thighs with CPC has the potential to retain similar levels of total aerobic bacteria regardless of the artificial inoculation of S. Infantis. In conclusion, the treatment of skin-on poultry parts with CPC may reduce the risk of foodborne outbreaks caused by Salmonella Infantis.

**Key Words:** Salmonella Infantis, cetylpyridinium, peracetic acid, skin-on parts, antimicrobial dips

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**269 Comparison of media for the detection of Campylobacter jejuni using a commercial RT-PCR system.** Aaron Bodie, Peter Rubinelli, April Englishbey, Savannah Forgey, Tyler Stephens, Steven Ricke, Food Science, University of Arkansas, Fayetteville, Arkansas, United States, Animal and Food Science, Texas Tech University, Lubbock, Texas, United States, 3Qualicon Diagnostics LLC, A Hygiena Company, New Castle, Delaware, United States.

Campylobacter jejuni is a major cause of diarrhea and foodborne gastroenteritis worldwide. Rapid and accurate detection of C. jejuni is needed to improve surveillance throughout food processing plants. The purpose of the current study was to compare the limit of detection for C. jejuni in different media using a commercial real time polymerase chain reaction (RT-PCR). C. jejuni ($10^6$ CFU/mL) was inoculated and grown for 24 h under anaerobic conditions in either a selective medium, Mueller-Hinton Broth (MHB); enrichment medium, Bolton’s blood free broth (BB); or Buffered Peptone Water (BPW). The experiment was repeated three times with three technical replications per medium. After 24 h, samples were
serially diluted to $10^{-5}$, 10 µL was spot plated in quadruplet on Modified Charcoal-Cefoperazone-Deoxycholate Agar (MCCDA), and subsequently incubated under microaerophilic conditions for 24 h at 42 °C. RT-PCR was performed in duplicate using the BAX®Q7 RT-PCR system. Data was statistically analyzed using a linear regression and considered significant when $P < 0.05$. Slopes of the linear regressions of each media was compared using an analysis of covariance (ANCOVA). The BAX®Q7 RT-PCR system was able to detect C. jejuni regardless of media used. BPW had the highest limit of detection (3.03 Log$_{10}$CFU/mL), while MHB and BB reached a lower limit at 2.56 Log$_{10}$CFU/mL and 2.93 Log$_{10}$CFU/mL, respectively. Using the designed primers and PCR assay of the BAX Q7 RT-PCR system, the amplification efficiency of all media was above 90%, while standard curves developed had an $R^2 \geq 0.87$ and RMSE $\leq 0.58$. The result of the ANCOVA was $P > 0.05$, proposing there is no significant difference between the slopes of each standard curve. The results of this study suggest that all media types (MHB, BB and BPW) were effective in detecting C. jejuni using the BAX®Q7 RT-PCR system. This RT-PCR assay and system will provide rapid and sensitive approach for the detection of C. jejuni for poultry processors and food safety personnel.

Key Words: C. jejuni, detection, RT-PCR, media

270 Effect of plant derived antimicrobials in disrupting horizontal transfer of antibiotic resistance gene between multidrug resistant Salmonella and commensal E. coli in broilerchicks ex vivo and in vivo. Poonam Vinayamohan$^{\text{a,b}}$, Abraham J. Pellissery$,^\text{a}$, Oliver R. Perrine$,^\text{a}$, Atacan Zeran$,^\text{a}$, Annie M. Donoghue$,^\text{c}$, Kumar Venkitanarayanan$,^\text{a}$, $^\text{a}$Department of Animal science, University of Connecticut, Storrs, Connecticut, United States, $^\text{b}$Poultry Production and Product Safety Research Unit, ARS, USDA, Fayetteville, Arkansas, United States.

Bacterial horizontal gene transfer (HGT) represents the major route for dissemination of antibiotic resistance (AR) in the environment. The poultry gut is considered the “melting pot” of HGT due to the dense and diverse nature of the enteric microbial populations and the protection they provide to DNA during transfer. Therefore, strategies that inhibit bacterial conjugation (primary mode of HGT) between AR bacteria and commensal bacteria could potentially reduce the dissemination of AR genes. Salmonella Heidelberg (SH) has emerged as one of the common serovars associated with foodborne infections in humans, especially poultry-derived foods. This study investigated the efficacy of two plant-derived antimicrobials (PDA) namely transcinnamaldehyde (TC), carvacrol (CR), and their combination in reducing the HGT of β-lactamase gene (blaTEM) between multidrug resistant SH to a nonpathogenic E. coli strain in broiler chicks. Day-old Cornish cross broiler chicks were randomly allocated to seven treatment groups from day of arrival, including untreated negative control, HGT positive control, two levels of TC (0.5% and 1%), two levels of CR (0.125% and 0.5%), and a combination of TC (0.25%) and CR (0.25%). In the ex-vivo study, on day 6, five birds from each group were euthanized to collect ceca to perform HGT inhibition study. The ceca were individually inoculated with 8 log CFU of donor SH (ampicillin-streptomycin resistant) and recipient E. coli (nalidixic acid-streptomycin resistant). After 4 h of incubation at 41°C, HGT frequency was determined from the transconjugant population (ampicillin-nalidixic acid-streptomycin resistant). In the in vivo study, on day 8, the remaining treatment groups except the untreated negative control were orally gavaged with 8 log CFU each of donor SH and recipient E. coli. On days 10 and 12, eight birds from each group were sacrificed and cecal contents were analyzed for HGT frequency, as previously mentioned. All the data were analyzed using one-way ANOVA and corrected for multiple comparisons using Dunnett’s multiple comparison test. In the ex vivo study, 1% TC, 0.125% CR and the combination treatment (0.25% TC and 0.25% CR) for seven days reduced the HGT frequency of blaTEM gene from SH to E.coli by 90% compared to control (p<0.05). Moreover, 0.5% TC and 0.5% CR decreased HGT frequency by 50% (p<0.15 - p<0.17). Furthermore, the in vivo conjugation experiments also revealed more than 20% reduction in HGT frequency on day 10 and day 12 (p<0.4 - p<0.9) compared to control. Collectively, the data suggest the potential effect of PDAs in reducing HGT and AR bacteria in poultry, thereby improving food safety.

Key Words: Antibiotic resistance, poultry, plant derived antimicrobials, horizontal gene transfer, Salmonella

271 Plant-derived compounds, eugenol, carvacrol, and beta-resorcylic acid reduce Salmonella on chicken wings at scalding and chilling conditions. Divek V. T. Nair$^{\text{a,b}}$, Shijinaraj Manjankattil$^\text{a}$, Claire Peichel$^\text{a}$, Annie M. Donoghue$^\text{c}$, Kumar Venkitanarayanan$^\text{a}$, Anup Kollanoor Johny$^\text{a}$, $^\text{a}$Department of Animal Science, University of Minnesota, Saint Paul, Minnesota, United States, $^\text{b}$Poultry Production and Product Safety Research, University of Arkansas, Fayetteville, Arkansas, United States, $^\text{c}$Department of Animal Science, University of Connecticut, Storrs, Connecticut, United States.

Non-typhoidal Salmonella outbreaks linked to poultry products necessitate effective and safe antimicrobial interventions during processing stages. In this study, the efficacy of three plant-derived compounds (PDCs), eugenol, carvacrol, and beta-resorcylic acid was evaluated against Salmonella on chicken wings during scalding and chilling conditions of poultry processing. Chicken wings were inoculated with a two-serotype mixture of Salmonella Enteritidis and S. Heidelberg (6 log$_{10}$ CFU/wing). After 30 min of Salmonella attachment at room temperature, each wing was treated with beta-resorcylic acid (BR; 0.5%, 1%), eugenol (EG; 0.5%, 1%), carvacrol (CR; 0.5%, 1%), chlorine (CL; 200 ppm), or peracetic acid (PA; 200 ppm). Wings inoculated with Salmonella subjected to no antimicrobial treatments served as the positive control (PC).
For scalding studies, the treatments were applied for 2 min at 54°C. Additionally, 0.5% of poultry droppings were added to the treatment water to simulate organic load during scalding. Separate wings were used for chilling studies, and the treatments were applied for 30 min at 4°C. The treated samples were homogenized in PBS for 45 s. Sample homogenates and treatment water were plated on xylose lysine deoxycholate agar and enriched in selenite cystine broth for determining the surviving Salmonella. Studies were repeated 6 times (6 wings/treatment), and data were analyzed using PROC-ANOVA (*P<0.05 considered significant). Under scalding conditions, all treatments, except CL, yielded significant reductions of Salmonella on wings compared to PC. Reductions were 0.6-, 1.0-, 0.9-, 1.7-, 1.6-, 3.3- and 1.4- log10 CFU/g, respectively for 0.5% BR, 1% BR, 0.5% EG, 1.0% EG, 0.5% CR, 1% CR and PA (*P<0.05). The largest Salmonella reduction of 3.3 log10 CFU/g was obtained with the application of 1% CR. All the treatments, except CL and PA, reduced Salmonella to non-detectable levels in the scal'd water (*P<0.05). Salmonella reductions on wings during chilling were 1.0-, 1.1-, 1.3-, 0.9-, 2.1-, 4.0- and 2.4- log10 CFU/g for 0.5% BR, 1% BR, 0.5% EG, 1.0% EG, CL, 0.5% CR, 1% CR and PA, respectively. A maximum reduction of 4.0 log10 CFU/g in Salmonella was observed for 1% CR. All treatments, except BR and CL, resulted in non-detectable Salmonella in chilling water (*P<0.05). The study revealed that, PDCs could be effective and safe antimicrobials against Salmonella on poultry products, however, follow up sensory studies are needed (University of Minnesota-ARS agreement#59-6022-7-002 of the USDA-OREI Grant#2017-51300-26815).

Key Words: Salmonella, Plant-derived compounds, Scalding, chilling, Antimicrobial

272 Effect of plant-derived antimicrobials against drug-resistant Campylobacter jejuni in turkey poulets.
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Drug-resistant Campylobacter is an emerging concern in turkey production. Research is required to develop environmentally friendly, natural solutions against multidrug-resistant (MDR) Campylobacter before the pathogen becomes a frequent problem in the turkey food supply. In this study, we tested two plant-derived compounds, trans-cinnamaldehyde (TC) and thymol (TH) against an MDR strain of Campylobacter jejuni BEAR-11504 (CJ; source-turkeys; resistant to tetracycline, erythromycin, and azithromycin) in turkey poulets. Two independent studies were conducted. Each study followed a completely randomized design with five treatment groups (N=40), each with 8 poulets. Day-old poulets were challenged with 3 - 4 log10CFU of MDR CJ and distributed to one of five groups: TC (challenged with MDR CJ, and TC supplemented at 0.06% and 0.08% in study 1 and 2, respectively, in water), TH (challenged with MDR CJ, and TH supplemented at 0.25% and 0.5% in study 1 and 2, respectively, in water), and positive control (PC – challenged with MDR CJ and neither TC or TH). Birds that received no intervention and not challenged with MDR CJ were kept as the negative control (NC). After 48 hours of inoculation, birds were euthanized, and cecal samples were collected in phosphate buffer saline for MDR CJ recovery. Cecal samples were homogenized, serially diluted, and plated on Campy Cefex agar. Enrichment of cecal samples for CJ growth was done in Bolton broth. Bacterial colonies were identified based on morphology and confirmed using the Campylobacter agglutination kit. Bacterial counts were log-transformed and analyzed for statistical significance using the SAS program (*P<0.05). Birds in the NC group remained negative for CJ throughout the study. In study 1, therapeutic supplementation of 0.06% TC and 0.25% TH in water reduced the CJ populations in poult cecum by 0.7 log10CFU/g but was not significantly different from the PC. In study 2, a significant reduction of 1.2 log10CFU of MDR CJ/g of the cecum was found with supplementation of TC (0.08%) through drinking water in poult's compared to PC (P<0.005). The results show that TC could be a potential plant-based intervention to control drug-resistant Campylobacter in turkeys. However, more studies are warranted to validate its efficacy in turkey production and processing (Funding by the CFANS Bridge and Development Grant, University of Minnesota).

Key Words: Drug-resistance, Campylobacter jejuni, trans-cinnamaldehyde, thymol, antibacterial

273 Efficacy of caprylic acid, caprylic acid and cinnamaldehyde in reducing Salmonella Heidelberg on chicken eggs. Abraham J. Pellissery*, Poonam Vinayamohan, Oliver R. Perrine1, Atacan Zeran1, Abhinav Upadhyay1, Annie M. Donoghue1, Kumar Venkitanarayanan1, Animal Science, University of Connecticut, Storrs, Connecticut, United States, 2Animal science, University of Connecticut, Storrs, Connecticut, United States, 3Poultry Production and Product Safety Unit, ARS-USDA, Fayetteville, Arkansas, United States.

Salmonella enterica serovar Heidelberg (SH) is a significant foodborne pathogen causing human outbreaks primarily linked to poultry-derived foods. Besides its high invasiveness in the host, SH is resistant to multiple antibiotics, including aminoglycosides, fluoroquinolones and beta-lactams. Chicken eggs can get contaminated with SH from a variety of sources on the farm, highlighting the need for effective egg surface disinfection methods. This study investigated the efficiencies of a GRAS (Generally Recognized as Safe)-status compounds, caprylic acid (CAO), caprylic acid (CAY) and cinnamaldehyde (CUM), applied as a pectin-based coating for reducing SH on shell eggs. All three phytochemicals have proven efficacy as an antimicrobial agent, however, its efficacy in reducing
foodborne pathogens on eggs is unknown. Surface sterilized white-shelled eggs were spot inoculated with a 3-strain mixture of SH (8.0 log CFU/mL) and coated evenly with pectin-based CAO (1%), CAY (1%) and CUM (1%) treatments. In addition, a pectin-based coating containing a combination of all three phytochemicals (COMB) each at 0.5% v/v level of inclusion was also applied as a coating treatment on SH inoculated eggs. The treated eggs were stored at 4°C and SH populations enumerated on days 0, 1, 3, 5, 7, 14 and 21 of storage. The study was replicated twice (5 eggs/treatment/day), and data were analyzed using Student’s t-test with significance tested at p<0.05. On day 0, 8 log CFU/egg SH counts were recovered from control eggs coated with pectin. At the end of refrigerated storage (day 21), pectin-based of CAO and CAY at 1% level coating were effective in reducing SH on eggs, where the pathogen load was decreased by 2.0 log CFU/egg compared to controls on day 21 (p<0.05). In addition, 1% CUM pectin-based coating significantly reduced SH counts by 3.0 log CFU/egg compared to the untreated control on the last day of storage. Furthermore, combination treatment of all three phytochemicals (COMB) was most effective treatment wherein the SH count reduction ranged between 2.75-3.5 log CFU/egg from day 0 through day 14 and by the last day of storage, the counts were reduced by 3.7 log CFU/egg compared to the untreated controls (p<0.05). Results indicate the potential efficacy of the aforesaid phytochemicals in reducing SH on shell eggs; however, further studies investigating the industrial feasibility of their treatment and effects on sensory attributes of eggs are warranted.

Key Words: Salmonella Heidelberg, caproic acid, caprylic acid, cuminaldehyde, pectin-based coating

274 Withdrawn.
Physiology and Reproduction

275  Seminal characteristics of Creole roosters of Mexico in two seasons of the year. Rosalia Ordaz-Contreras1,2a, Fernando González-Cerón1, Arturo Pro-Martínez2, Josafat Salinas-Ruiz1, Said Cadena-Villegas1, Juan M. Cuca-García3, Jaime Gallegos-Sánchez2, Diego Zárate-Contreras1, Miguel A. Matus-Aragón1, Eliseo Sosa-Montes4, ‘Livestock Program, College of Postgraduates Campus Montecillo, Texcoco, State of Mexico, Mexico, 2College of Postgraduates Campus Cordoba, Cordoba, Veracruz , Mexico, 4College of Postgraduates Campus Tabasco, Tabasco, Mexico, 3Animal Science Department, Chapingo Autonomous University, Texcoco, State of Mexico, Mexico.

The objective of this research was to evaluate seminal characteristics of Creole roosters (Gallus gallus domesticus) of Mexico, during the summer and fall of 2019, to assess the effect of seasonal variation. Seventeen 35-week-old Creole roosters of a randombred experimental flock were used. Semen was collected every week by dorso-abdominal massage technique for fresh evaluation. Seasons of the year were the treatments (S: summer and F: fall) and the duration of the study was 10 weeks (W) in each season. Birds were kept in individual cages (60 cm x 60 cm x 60 cm) inside a poultry house with a light regime of 16 h. This house was naturally ventilated with adjustable curtains on the sides. Roosters were fed a diet with 17% CP and 2800 kcal of ME/kg of feed. The daily feed intake was 120 g animal-1 and water was provided ad libitum. Variables analyzed were: semen volume (SEVOL), percentage of live sperm (PLE) and sperm concentration (SCE). Data were analyzed using the GLIMMIX procedure of SAS with repeated measurements over time and an LSD mean test (P < 0.05). SEVOL was different (P < 0.0001) between seasons (S and F) at W5 (S: 0.24 ml vs. F: 0.33 ml) and W10 (S: 36 ml vs. F: 0.19 ml). PLE in semen samples showed differences (P < 0.0001) at W1 (S: 99.1% vs. F: 73.6%), W3 (S: 95.9% vs. F: 81.8%), W4 (S: 92.9% vs. F: 78.4%), W5 (S: 99.4% vs. F: 71.2%), W7 (S: 92.8% vs. F: 68.0%), W8 (S: 98.6% vs. F: 76.7%), W9 (S: 97.0% vs. F: 71.6%) and W10 (S: 84.6 vs. F: 66.6%). Differences were observed in SCE (P < 0.0001) at W3 (S: 1.78 x109/ml vs. F: 3.14x109/ml), W6 (S: 2.10x109/ml vs. F: 3.78x109/ml), W8 (S: 4.36x109/ml vs. F: 2.48x109/ml), W9 (S: 3.68 x109/ml vs. F: 1.53 x109/ml) and W10 (S: 4.03 x109/ml vs. F: 2.01x109/ml). In conclusion, Creole roosters have a better reproductive performace in terms of volume of semen, percentage of live sperm and sperm concentration.

Key Words: Creole hens, eggshell color, hatching, Mexico

276  Eggshell color and hatch-variables in eggs from Creole hens of Mexico. Leonardo Osio-Orihuela1,2a, José G. Reyes-Bello1, Osvaldo Fernández-Ipiña1, Diego Zárate-Contreras1, Arturo Pro-Martínez1, Juan M. Cuca-García1, Leodán T. Rodríguez-Ortega1, Fernando González-Cerón1, ‘Department of Animal Science, Chapingo Autonomous University, Texcoco, State of Mexico, Mexico, 2Livestock Program, College of Postgraduates Campus Montecillo, Texcoco, State of Mexico, Mexico, 3Polytechnic University of Francisco I. Madero, Francisco I. Madero, Hidalgo, Mexico.

It has been evidenced that eggshell color has an effect on hatching results in species and breeds of poultry. However, this information is unknown in Creole hens (Gallus gallus domesticus) of Mexico (CHMX). The objective of this study was to evaluate the effect of eggshell color on hatch-variables of eggs from CHMX. Seven hundred and nine eggs from 66 Mexican Creole hens, belonging to an experimental randombred flock, were collected and stored from 0 to 14 d (depending on the day the egg was laid) until incubation. Eggs were classified according to shell color (seven-categories from light brown to dark brown) and grouped in batches of five to six eggs each. All eggs were set in a single-stage incubator at 37.5 °C and 55 % relative humidity; eggs were turned once each hour. On 19 d of incubation eggs were transferred to a hatcher kept at 37 °C and 65 % relative humidity. At 12 d of incubation eggs were lamped individually to identify unfertile eggs and dead embryos. At hatching day (21 d of incubation), all unhatched eggs were examined to continue the determination of embryonic mortality. Egg examination data were used to estimate hatch-variables: percentage fertility (%FERT), percentage embryonic mortality (%EM) and percentage hatchability (%HATCH) per batch and eggshell color category. Hatch-variables data were analyzed by ANOVA with GLM procedure of SAS. Each batch of eggs per eggshell color constituted a replicate of the corresponding color category. Estimated means of %FERT, %EM, and %HATCH varied from 70.0 to 86.7; 47.3 to 66.7; and 10 to 36.7, respectively. No differences (P ≥ 0.05) were detected among eggshell color categories in terms of the analyzed hatch-variables. In conclusion, eggshell color of eggs from Creole hens of Mexico does not influence fertility, embryonic mortality or hatchability.

Key Words: Creole hens, eggshell color, hatching, Mexico

277  Broiler Breeder hen sexual maturity and hatching egg production influenced by a phytomolecules-based additive program. Twan van Gerwe1, Henning Gerstenkorn2, Ajay Bhoyar*1, Kowsigaraj Palanisamy1, ‘Technical Management, EW Nutrition, Visbek, Germany, 2Product Management, EW Nutrition, Visbek, Germany, 1Global R & D, EW Nutrition, Visbek, Germany.

To regulate weight gain, limit health risks, and maintain fertility, broiler breeders are fed restrictively. The resulting diurnal shift from a fed state to feed deprivation is likely to affect the birds’ physiology. In this experiment, two phytomolecules-based gut health additives were combined, applied through feed and water, from day-old till 50 weeks of age. The effect on onset of and production of hatching
eggs, bodyweight and uniformity, as well as fertility, hatchability and gut health was assessed. 1,560 Ross 308 day-old-chicks were randomly allocated in rearing pens. ‘Treatment’ birds received feed with the phytomolecules-based feed additive Activo® (@100g / MT) throughout and were supplemented with Activo® Liquid through the water in a scheduled application period, to support gut health during challenging episodes. ‘Control’ birds were not supplemented with these additives. Males and females were transferred to the production house at 19 and 20 weeks of age, respectively. Per treatment, 10 pens were populated, each housing 55 hens and 7 males. Ninety hatching eggs per pen were set at 7 different ages (range week 29-49). Fertility and hatch of fertile were assessed at ED10 and Day of hatch respectively. Treatment group attained 5% production 6.4 days (P<0.01) earlier than the control group. Hatching Egg production / Hen Housed was significantly higher in the treatment group (D 7.0, P=0.002). At 23, 27 and 44 w of age, a random female per pen was sacrificed, and breast muscle and abdominal fat pad were weighed, indicating heavier fat pads in ‘treatment’ than ‘control’ (P<0.01). At 44 w of age, villi height, crypt depth of jejunum was determined, indicating significantly (14%) increased villi height in Activo® + Activo® Liquid supplemented birds (P=0.08). Fertility and hatchability were high throughout the experiment for both groups, at 97.3% and 87.6% (P=0.08). From treatment for both groups, at 97.3% and 87.6% respectively. Consequently, DOC production per hen-housed till 50 weeks of age was ± 6 higher for Activo® + Activo® Liquid supplemented birds than control birds.

Key Words: breeder, additive, Gut, Egg, villi

278 Bone and Eggshell Quality Through an Extended Laying Cycle in Three Differentially Selected Strains of Laying Hens. Charlene Hanlon6,7, Kayo Takeshima, Gregory Y. Bedecarrats, Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada.

Decades of intensive genetic selection in commercial laying hens have resulted in early entry in lay, while sustaining high production rates up to 100 weeks of age (woa). To achieve this, substantial adaptations of the hen’s physiology are required, including calcium (Ca) metabolism to sustain eggshell formation while maintaining a healthy skeletal frame. Thus, we compared skeletal growth, bone and egg quality in 3 strains of layers with the Lohmann LSL-lite (L) as the current commercial strain, heritage Shaver white leghorn (S) as the mid-2000s strain, and white-leghorn derived Smoky Joes (SJ) as the non-selected 1960s strain. At 12 woa, pullets were randomly assigned per strain to cages (n=2/cage). Photoperiod was set at 10h until photostimulation at 18 woa (1h/week to 16h light). At 18 woa, all hens were switched from a grower (1.2% Ca) to a layer (4.1% Ca) diet. Tibia and Femur were collected at 12, 17, 20, 25, 45, 60, 75 and 100 woa (n=4/strain). Bones were measured and weighed, and mineral density was assessed within the medullary (mBMD) and cortical (cBMD) regions of the tibia using microCT. Egg analyses, including weight, width and length, along with eggshell thickness (EST) and eggshell breaking strength (EBS), were conducted at 26, 40, 60, 70, 80, 90 and 100 woa. Statistical analyses were performed using the MIXED procedure in SAS with Tukey’s adjustment. Femur and tibia weight or size did not increase after 12 woa in any strains, indicating that all hens had reached maximum skeletal size at this time. While cBMD was not found to have an interaction with age and strain, a significant interaction (P=0.0053) was observed for mBMD. In SJ, mBMD increased throughout the laying period, while for S, it increased at the initiation of lay (20 woa), decreasing at peak of lay (25 woa), before increasing from 45 to 100 woa. Meanwhile, mBMD in SJ only increased by 75 and 100 woa. Overall, with no decline in cBMD or mBMD during the extended laying period, no detrimental effects on the tibia were observed. Egg weight increased over time for L and S (P<0.001). Increased egg weight resulted in a decline in EST and EBS over time (P<0.01), although these parameters were the highest for L eggs at peak of lay (26 woa) compared to S and SJ (P<0.01). Egg weight for SJ increased between 26 and 40 woa, remaining constant thereafter. By the end of the study, none of the strains differed in EST or EBS despite their varied production rates. In conclusion, our results show that although genetic selection of layer hens resulted in tremendous improvement in productivity, this did not impact the cBMD or mBMD in the tibia, suggesting a retained skeletal health through 100 weeks of life.

Key Words: Laying Hen, Egg Quality, Bone Quality, Calcium, Reproduction

279 Ovarian auto- and allotransplantation in turkeys (Meleagris gallopavo) to evaluate surgical techniques and graft rejection. George B. Hall1,2,7, Julie A. Long1, Ben J. Wood5,6,7, Gregory Y. Bedecarrats1, ‘Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, ‘Beltsville Agricultural Research Center USDA, Beltsville, Maryland, United States, ‘Hybrid Turkeys, Kitchener, Ontario, Canada, ‘School of Veterinary Science, The University of Queensland, Gatton, Queensland, Australia.

Biobanked turkey ovaries could theoretically be revived through transplantation but surgical protocols first should be evaluated using fresh tissue. In this study, we evaluated if successful attachment and size of the graft was affected by the amount of recipient ovary remaining or placement of the donor tissue. The grafts were also analyzed to determine whether they were being immunologically rejected. For this study, recipient poults were 1-2 days old while donors were 7-9 days old. Five experimental groups were evaluated.
Groups 1-4 were allotransplants receiving two pieces (4 mm² each) of donor ovarian tissue. For Groups 1, 2 and 3, the amount of recipient’s ovary removed was 33%, 66%, and 100%, respectively, and the donor tissue was placed orthotopically. For group 4, 100% of the recipient’s ovary was removed but the donor tissue was placed orthotopically and under the abdominal air sac membrane. Group 5 was an autotransplant group, where two pieces (4 mm² each) of the recipient’s ovary were immediately re-transplanted in the same location following 100% removal. Recipients were euthanized 6 days after surgery, and all transplants were photographed and fixed for analysis. Fixed ovaries were sectioned and stained (H&E) or processed for immunohistochemistry (IHC) against T and B lymphocyte markers, CD3 and MUM-1, respectively. Photos and H&E slides were used to determine the presence and size of grafts. The IHC slides were analyzed using ImageJ (Deconvolution) to determine the percent of positive cytoplasmic (CD3) and nuclei (MUM-1) area. A one-way ANOVA, followed by a Tukey post-hoc test was used to evaluate differences between surgery groups for graft size, attachment was 91% (32/35), with the average size of allo- and autotransplants being 4.2 ± 0.6 mm². There was no difference (P > 0.05) in the success rate or graft size between surgery groups. Allotransplants exhibited a higher percentage (P ≤ 0.001) of CD3-positive cytoplasmic area (49.78 ± 3.90%) than autotransplants (0.10 ± 0.03%). Similarly, allotransplants exhibited more (P ≤ 0.001) MUM-1 positive nuclei area (9.85 ± 1.95%) than autotransplants (0.34 ± 0.15%). The results showed that the portion of the recipient ovary left behind did not affect the rate of attachment or the amount of donor tissue that attached. Placing the donor tissue under the abdominal air sac did not affect success. Importantly, allotransplants were found with high proportions of T and B lymphocytes. From these results, we recommend removing all of the recipient’s ovary before transplanting tissue and medicating pouls with an immunosuppressant to halt rejection.

Key Words: Ovary, Allotransplant, Autotransplant, Immunology, Turkey

280 Potentials of physiological parameters to elucidate the internal development of early chick embryos. Minoru Takehara¹, Masaya Kishi¹, Atsushi Asano², Atsushi Tajima³, ¹Grad. School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan, ²Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan.

Commonly used criteria to determine the developmental stages of chick embryos was developed by Hamburger and Hamilton (1951). In this method, developmental stages of newly laid eggs (day 0) until hatching (day 21) are subdivided into 48 stages based on the phenotypic changes of embryos proper. It has been suggested, however, that the pace of internal organ development do not necessarily synchronize with the phenotypic changes of embryo proper.

In the present study, therefore, an attempt was made to detect internal organs development of chick embryos by using physiological parameters. One hundred fifty fertilized White Leghorn eggs were incubated in an air forced incubator. At 5, 6, 7, 8, and 9th days of incubation, 30 eggs were used to collect blood from blood vessel under inner shell membrane, and major blood chemical components were analyzed. DNA was isolated from blood cells and sex of embryos were identified by PCR using primer designed to identify W chromosome specific sequence. After the blood collection, embryo proper was exposed, and the body dimensions and weight of major visceral internal organs were measured. A completely randomized design with two main factors (sex and days of incubation) was used to analyze data and Tukey method was used to compare means between main effects and their interactions. Significant decreases were observed for weight of embryo proper, liver/ body weight ratio and stomach / body weight ratio throughout the observation period. On the other hand, heart / body weight ratio remained between 0.8-1.0 %, blood pH ranged between 8.1-8.2, and hematocrit were fluctuated between 21 – 26 %. Among blood components, a significant increase was observed for blood protein, cholesterol, fatty acids and phospholipids, whereas significant decreases were observed for Ca2+ and Mg2+ concentrations. Little changes were observed for glucose and inorganic phosphate concentration. In summary, significant correlations were observed between dimensions of visceral organs and concentrations of several blood components during the embryonic development. Present results indicate that physiological parameters could be used to detect the internal development of early chick embryos.

Key Words: Chick, Embryo, Development

281 Fertile hatching eggs response after treatment of pulsed ultraviolet light. Lindsey Bright¹,², Joshua Cassar², Paul Patterson², Edward Mills³, Ali Demirci¹, ¹Ag & Biological Engineering, Penn State University, University Park, Pennsylvania, United States, ²Animal Science, Penn State University, University Park, Pennsylvania, United States.

Every year the poultry industry in the United States sets over 14.2 billion eggs in hatchery incubators that produce the chicks destined for the egg, meat bird and other industries. When incubating eggs, maintaining a low microbial presence is crucial for ensuring biosecurity and good chick health. Pulsed ultraviolet light is a non-thermal, non-aqueous, antimicrobial intervention that has been reported to achieve a range of microbial reduction on the surface of eggs that could benefit the hatchery industry. This study evaluated the effects of UV light treatment on both embryo and chick health. Using a modified egg candling conveyor with a xenon flashlamp positioned parallel to the long axis of the conveyor, 4 replicates of 125 fertile eggs were treated with 0 (control), 10, 50 or 100 J/cm² of PUV light. All eggs used during this study came from 48-week-old Lohmann
LSL-Lite breeder hens. After PUV light treatment, eggs were placed in a commercial incubator and incubated under normal incubation conditions. The impact of treatment on chick weight was determined using Proc-GLIMMIX, while fertility, hatchability and livability percentage data were arcsin transformed and evaluated using the Proc-GLM procedures of SAS 9.4. There was no significant effect of the PUV light treatment on fertility or hatchability with averages of 92.9% and 87.8%, respectively (p>0.05). Furthermore, there were no significant effects on post-hatch observations, including livability and average bird body weight at 1, 7, and 42 days of age, which were 39.6, 69.4, and 471.6 g, respectively (p<0.05). This study suggested that treatment of hatching eggs with PUV light as a surface antimicrobial intervention has no negative effects on egg fertility, hatchability, or chick livability and body weight.

**Key Words:** Pulsed, ultraviolet, light, hatching, eggs

282 Expression of umami receptors (T1R1/T1R3) in the intestine during the embryonic and post-hatch development in broiler chickens. Bingqi Dong, Emily Kim, Elijah Kiarie, Chengbo Yang, Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada; Animal Biosciences, University of Guelph, Guelph, Ontario, Canada.

Umami receptors T1R1/T1R3 can recognize protein breakdown products including amino acids and peptides. Our previous results demonstrated that umami receptors T1R1/T1R3 are widely expressed in both the oral cavity and intestines of broiler chickens. Specifically, the jejunum had the highest expression level of umami receptors within the intestine. However, the gene expression of umami receptors T1R1/T1R3 in the intestine during the embryonic and post-hatch development in chickens is still unknown. This study was to investigate the gene expression of T1R1/T1R3 in the intestine during the late embryonic and post-hatch development in broiler chickens. A total of 15 fertilized broiler eggs and 20 healthy broilers were used in this study. The jejunum was collected from 3 embryonic broiler chickens at the embryonic day (E) 17, 18, 19, 20 and 21. Four chickens were randomly sacrificed at 1 week, 2 weeks, 3 weeks, 4 weeks, and 5 weeks, and duodenum, jejunum, ileum, and colon were collected. We found the T1R1/T1R3 mRNA abundance was detected in the jejunum at all stages of the late embryonic development. The jejunal T1R1/T1R3 mRNA abundance had increased with age during the late embryonic development in chickens. The expression of T1R1/T1R3 in the duodenum, jejunum, ileum, and colon of chicks of different ages was varied. These results demonstrated that chicken umami receptors T1R1/T1R3 are expressed in the intestine in the late embryonic stage and post-hatching stage and their levels of expression may be affected by the ages of embryonic development and post-hatch development. However, the roles of chicken umami receptors T1R1/T1R3 in the intestine are still not clear. Further studies are needed to investigate the potential ligands of chicken umami receptors and cell signaling pathways activated by potential umami substances.

**Key Words:** chicken umami receptor T1R1/T1R3, gene expression, intestine, gut chemosensing, chickens

283 The impact of the sound shock of the embryonic development and physiological traits in chicks embryo. Salwan Abdulateef, Ziyad T. Aldhanki, Thafer Mohammed, Animal Production, University of Anbar, Ramadi, Iraq.

The neural response of chicks begins during the development of nerves and the formation of the brain. At this stage, chicks recognize external stimuli such as sound, light, and heat...etc. hens incubate eggs and release sounds that chicks inside eggs can feel. Chicks gain comfort from hen's feeling. However, this is missing in the artificial hatchery. Therefore, in the current study, hatcheries were provided with different sounds as external stimuli. The neural and physiological responses of chicks were investigated at the Department of Animal Production, College of Agriculture, University of Anbar, Iraq. 300 eggs (Ross 308) were allocated in four treatments as follows: control without shock (CW), low sound shocked (10) dB (LS), medium sound shocked (30) dB (MS), high sound shocked (75) dB (HS). Different sound devices were used to shock the eggs three times a day from day 11 to 21 days. This experiment was carried out by using Complete Randomized Design (C.R.D), and the Data were analyzed by using the SAS program for statistical analysis (SAS). The means for each treatment were compared by using Duncan's polynomial with 0.05 and 0.01 significance level to determine the significant differences between the averages. A significant increase (P<0.01) in the percentage of embryonic weight and amniotic sac and liquid was observed. Moreover, a significant decrease (P<0.01) in the percentage of albumin and yolk compared to the control treatment at 14 days of incubation for experimental treatment LS and MS. A significant increase (P<0.01) in the proportion of embryonic weight and important decline (P<0.01) in the proportion of yolk at 17 days of incubation were observed under the sound treatments. A significantly increased (P<0.01) in neurophysiological traits of neurons (histological traits) under LS and MS treatments were reported. It also significantly increased (p<0.01) in brain weight for LS and MS compare with HS and CW. In concluding, the use of natural sound shock (low and medium sound shocks) in the embryonic period helped the development of embryos and improved the neurophysiological traits.

**Key Words:** sound, shock, embryonic, physiological, chicks

284 Delayed post-hatch feeding induces long-term alterations in intestinal barrier and nutrient transport function in broilers. Sofia Biakowskii, Liang-en Yu, Andrea Toschi, Yihang Li, Animal and Food Sciences,
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Adverse environment during early life alters the developmental trajectory of many organ systems, which leads to increased disease risk throughout the lifespan. Altered intestinal functions, such as barrier and nutrition transport, have been implicated as a mechanism in impaired health and growth performance in agricultural animals. However, the effect of early life stress on the intestinal development and function in poultry has not been fully elucidated. Delayed feeding is a common early life stressor in poultry production which negatively impacts performance and health. The objective of this study was to evaluate the impact of delayed post-hatch feeding in broilers on later-life intestinal permeability and electrogenic nutrient transport function. A total of 200 Ross 308 birds were hatched and allotted into either delayed feeding group (DF, no feed until ~72h post-hatching) or normal feeding group (NF, immediate access to feed). DF and NF chicks were co-housed and had ad libitum access to the same commercial diet till 42 d of age. Body weight (BW), feed intake (FI), and feed:gain ratio were recorded weekly. At 20 d and 41 d of age, oral FITC-dextran (FD4) gavage was conducted to evaluate permeability of the whole GI tract. At 21 d and 42 d of age, mid-jejunum was harvested and mounted on Ussing chambers to measure transepithelial resistance (TER), tissue specific FD4 permeability, and Na⁺-dependent nutrient transporter function, determined as the change in short-circuit current ([endif]→-[Isc]) in response to selected luminal nutrients (glucose, alanine, and glutamate). Student’s t-test was performed using GraphPad and significance denoted at P ≤ 0.05. In contrast to suppressed BW and FI (P < 0.05), feed:gain ratio in DF birds was numerically lower (P < 0.1) than control. Compared with age-matched NF birds, DF birds exhibited reduced jejunal TER (by 33%, P < 0.05) as well as increased permeability (by 80%, P < 0.1) to FD4 in jejunum at d21, but not at d42. In DF chickens, the whole GI tract was constantly leakier to FD4 at d21 and d42 (by 36% and 71%, respectively, P < 0.05) than that in NF birds. The jejunal Na⁺ glucose linked transporter (SGLT1) function was upregulated in DF birds (by 1.2-fold, P < 0.05) at d21 but not d42. Surprisingly, DF birds exhibited upregulation in amino acid transporter function for alanine and glutamate (by ~1.3-fold and 1-fold, respectively, P < 0.05) at d42 but not at d21. Together, the results indicated that early feed deprivation in broilers induces lasting permeable GI tract. The later-life upregulated nutrient transporter function in jejunum may be a reflection of increased nutrient demand in the stressed birds.

Key Words: delayed feeding, intestine, permeability, nutrient transport, broilers

Increased pre-prandial ghrelin concentrations have been reported in all mammals studied up to date. However, there are very few and conflicting reports on ghrelin concentration during fasting and refeeding in avian species. Therefore, we evaluated the effect of short term feed restriction and refeeding on concentrations of ghrelin, glucose, insulin, corticosterone and growth hormone (GH). Eleven one-day-old chicks were reared using normal feeding and lighting management as recommended by the industry. At 2 weeks of age (WOA), birds were weighed and placed in individual cages for adaptation. From 3 to 9 WOA (end of experiment: Weeks), feed was removed once a week from the cage and birds were fasted for 18 h. After fasting, a blood sample (Time 0; T0) was obtained from each bird. Then, all birds were fed, and a second blood sample (Time 1; T1) was obtained at 90 ± 5 min after feeding. Feed intake was measured by recording the weight of feed offered minus the unconsumed feed at 1.5 h after feeding. Blood samples were obtained at T0 and T1 weekly from 3 WOA – 9 WOA. Data obtained was analyzed as a completely randomized design with repeated measurement over time (PROC GLIMMIX, SAS). Feed intake (P < 0.001) at 1.5h after feeding between 3 - 9 WOA were different. There was no Time or Time x Week interaction for ghrelin concentrations. However, there was a Week (P< 0.001) effect on ghrelin concentration. Concentration of ghrelin were increased on week 9 (274.7 ± 17.4 pg/mL) as compared to all other weeks (205.7± 17.2 pg/mL). There was a Week (P < 0.001) and Time (P = 0.003) effect on glucose concentration. Glucose concentrations were decreased during T0 (167.1 ± 2.4 mg/dL) as compared with T1(179.9 ± 3.2 mg/dL). Additionally, glucose concentration increased during weeks 6 and 7 as compared with week 3. A Time x Week interaction was found for GH (P = 0.06), insulin (P = 0.01) and corticosterone (P = 0.05). Concentrations of GH were higher during T0 (2.8 ± 0.7 ng/mL) as compared with T1 (2.5 ± 0.7 ng/mL) only on week 3. Insulin concentrations were higher during T0 (13.9 ± 0.3 µIU/mL) as compared with T1 (12.5 ± 0.3 µIU/mL) only on week 4. Concentration of corticosterone were higher during T0 (95.8 ± 9.2 ng/mL) as compared with T1 (50.2 ± 9.7 ng/mL) on week 3. Taken together, fasting and refeeding was associated with a normal physiological response to glucose concentrations. However, the pre-prandial surge of ghrelin concentrations reported in mammalian species was not observed in male broiler chickens.

Key Words: Ghrelin, Broilers, Hormones, Fasting, Refeeding

The Effect of a ghrelin receptor agonist (capromorelin) on Feed and Water Intake, Body Weight Gain and Animal Behavior in Female Broad Breasted White Turkeys (Meleagris gallopavo). Katherine P. Sanmartin, Elisabeth Embden, Tameka Smith, Katherine P. Sanmartin, Elisabeth Embden, Tameka Smith, Katherine P. Sanmartin, Elisabeth Embden, Tameka Smith, Katherine P. Sanmartin, Elisabeth Embden, Tameka Smith.
Sanmartinosta1, Natalia Ceron-Romero1, Nurudeen O. Taofeek1, Ethan Vroonland1, Logan Swanson1, Martha Verghe1, Ernst Heinen1, Jorge Vizzcarra1, Food and Animal Sciences, Alabama A&M University, Huntsville, Alabama, United States, 1ELANCO, Greenfield, Indiana, United States.

In turkeys, ghrelin is a 28 amino acid peptide produce mainly in the proventriculus. We previously reported that short- and long-term feed restriction decreases ghrelin concentrations in turkeys and passive immunization against ghrelin increases feed intake. Thus, the opposite effects of that reported in mammalian species. We also reported that, similarly to dogs and cats, capromorelin (a ghrelin receptor agonist; Elanco®) increases feed intake in broiler chickens. The purpose of the present study was to evaluate the effect of capromorelin on feed intake, water intake, body weight (BW) gain and animal behavior in female turkeys. One-day old white female turkeys were obtained from a commercial hatchery. Birds were reared as recommended by the poultry industry. At 3 weeks of age turkeys were individually caged. After 3 days of adaptation to the cages (experimental day 0; D0), birds were blocked by BW (average ± SE; 650 ± 25 g) and randomly assigned to two treatments (0 or 12 mg/kgBW/day) of capromorelin in drinking water (n = 9/trt). Feed intake (FI) and water intake (WI) were measured three times a day at 0800h (Period 1; P1), 1200h (P2) and 1700h (P3), whereas BW was recorded daily during five experimental days (D0-D5). Feed and water intake was measured by recording the weight of feed or water offered each time minus any unconsumed feed or water remaining. Based on these data (i.e. WI and BW), the medicated water concentration was adjusted daily. To ensure that birds had sufficient medicated water for a 24 h period, the amount of water that was offered to birds per day was calculated based on the amount consumed the previous day times a factor of 1.3. Control birds received a placebo solution provided by the company. Bird behavior associated with feeding, seating and standing activity was evaluated for eight consecutive hours on D5 (n = 2/trt) using Ethovision software. Data on FI, WI and BW was analyzed using a randomized complete block design (RCBD) with repeated measures over time. Behavioral data was log transformed and analyzed as a RCBD. There were no significant treatment effects on FI (98.8 ± 5.4 g/d), WI (170 ± 9 mL/d) and ADG (49.2 ± 2.1 g/d). However, during P1 (i.e. 0800 to 1200) per hour FI (7.0 ± 0.5 g/h) and WI (10.1 ± 0.5 g/h) were higher as compared with P3 (P < 0.001). There was no significant treatment effect on seating and standing activity. However, turkeys that received capromorelin tended (P = 0.11) to have more activity around the feeder as compared with control birds. We concluded that in contrast to mammalian species and chickens, capromorelin does not increase FI or BW gain in turkeys.

Key Words: Ghrelin, Agonists, Feed Intake, Capromorelin, Turkeys

The effect of corticosterone infusion on the concentrations of insulin, ghrelin and glucose in full fed and feed restricted birds (Gallus gallus domesticus). Natalia Ceron-Romeroosta2, Nurudeen O. Taofeek, Katherine P. Sanmartin, Aulisea Thomas, Ethan Vroonland, Martha Verghe, Jorge Vizzcarra, Food and Animal Sciences, Alabama A&M University, Huntsville, Alabama, United States.

The understanding of appetite regulation in birds is vital to improving the efficiency of poultry feeding systems. Glucocorticoids have been reported to regulate both insulin and ghrelin secretion and therefore influence energy metabolism and appetite. In mammals, the orexigenic effect of ghrelin on appetite is widely reported. However, in birds, information on the regulatory effect of ghrelin on appetite is less understood. To the best of our knowledge, information on the effects of glucocorticoids on plasma concentrations of insulin, ghrelin and glucose in birds have not been reported. Therefore, the study was designed to evaluate the effects of corticosterone (CORT) infusion on ghrelin, insulin, and glucose concentrations in birds. One-day-old male broiler chickens (n = 24) reared according to poultry industry standards were enrolled. At two weeks of age (WOA), birds were individually caged, and randomly assigned to a 2 x 4 factorial arrangement of treatments. The first factor (FEEDING) consisted of feed restricted (RES) or full fed (FF) birds. The second factor (DOSE) comprised four doses of CORT (0, 100, 200, 400 µg/kg BW) given as IV boluses. Jugular cannulation was performed when birds weighted 2.0 ± 0.5 Kg to facilitate bolus treatment infusion and frequent blood collection (4 to 7 WOA). Hourly blood samples were obtained for six consecutive hours during three periods (BEFORE, DURING and AFTER; PERIOD). The first blood sample was collected before RES birds were fed. Data was analyzed as a completely randomized design with a factorial arrangement of treatments and repeated measurement over time using PROC GLIMMIX. Concentrations of ghrelin and insulin were measured only for the 400 µg/kg BW dose of CORT infusion. There was a FEEDING x DOSE x PERIOD interaction (P = 0.001) on CORT concentrations. As expected, CORT significantly increased DURING infusion (349 ± 82 ng/mL) as compared with BEFORE (14.6 ± 3.8 ng/mL) and AFTER (59.7 ± 15.3 ng/mL) CORT infusion. There was a PERIOD x DOSE interaction on glucose concentrations (P < 0.001). Concentrations of glucose increased DURING CORT infusion (221.0 ± 6.0 mg/dL) as compared with BEFORE (205.2 ± 6.2 mg/dL) and AFTER (265.7 ± 38.4 mg/dL) CORT infusion. However, there were no significant effects of treatments on insulin concentrations (22.2 ± 2.1 µIU/mL). There was a FEEDING x PERIOD effect on ghrelin concentrations (P < 0.001). Plasma ghrelin concentration in RES birds was significantly higher BEFORE (106.9 ± 7.8 pg/mL) and decreased DURING (76.1± 8.13 pg/mL) and AFTER (76.9± 8.4 pg/mL) infusion of CORT. We concluded that CORT infusion increased glucose concentration, and decreased ghrelin concentrations in male broilers chickens.
Key Words: Ghrelin, Corticosterone, Appetite, Endocrinology, Energy Metabolism

288 Effect of hot and cold temperatures on the proliferation and differentiation of pectoralis major muscle satellite cells from a commercial turkey line. Jiahui Xu1(✉), Gale M. Strasburg2, Kent M. Reed3, Sandra Velleman1, 1Department of Animal Sciences, Ohio State University, Wooster, Ohio, United States, 2Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan, United States, 3Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, Minnesota, United States.

Commercial poults immediately after hatch are inefficient at regulating their body temperature due to immature thermoregulation. However, newly hatched poults may be confronted with both hot and cold temperatures during handling and transport. Temperature extremes may result in long-term effects on muscle growth, meat yield and quality. Satellite cells (SCs), the main progenitors for muscle growth after hatch, have maximal mitotic activity and sensitivity to temperature stimuli during the first week of age. Previous studies have shown that SCs from growth-selected turkey pectoralis major (p. major) muscle are more sensitive to temperature stimuli. The objective of this study was to determine how cold and hot temperatures affect the proliferation and differentiation of SCs from the p. major muscle of 1-wk old Nicholas commercial (NC) turkeys selected for increased growth rate and meat yield as compared to 1-wk old Randombred Control Line 2 (RBC2) turkeys that represent commercial turkeys from 1966. Three temperature regimens were performed: SCs proliferated at 38°C but differentiated at 43° or 33°C; SCs proliferated at 43° or 33°C but differentiated at 38°C; and SCs proliferated and differentiated at 43°, 38°, or 33°C. DNA concentration, creatine kinase activity, and gene expression were measured by Hoechst 33258 fluorochrome, 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. At 72 h of proliferation, proliferation was increased at 43°C (P < 0.01) and decreased at 33°C (P < 0.01), with SCs from NC line having greater proliferation than the RBC2 line at 38°C (P < 0.01) and 43°C (P < 0.01). At 72 h of differentiation, SCs treated by thermal challenge during proliferation and/or differentiation showed increased differentiation at 43°C (P < 0.01) and decreased differentiation at 33°C (P < 0.01). When the thermal challenge was confined to proliferation, greater stimulatory or suppressive effects were observed for differentiation than in thermal challenges that occurred only during differentiation. At 72 h of proliferation, expression of myogenic determination factor1 and myogenin increased at 43°C (P < 0.01) in both cell lines, and SCs from the NC line were more sensitive to 43°C (P < 0.01) than those from the RBC2 line. These results suggest that elevating temperature during proliferation and/or differentiation can increase p. major muscle growth potential, and modern commercial turkey SCs are more sensitive to both hot and cold temperatures with the potential for impacting breast muscle growth and yield.

Key Words: temperature, turkey, satellite cell, proliferation, differentiation

289 Brain derived neurotrophic factor and extra-hypothalamic corticotropin releasing hormone neurons in the nucleus of hippocampal commissure play functional roles in the avian neuroendocrine regulation of stress. Hakeem J. Kadhim1(✉), Seong W. Kang1, Wayne J. Kuenzel1, 1Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, 2Cell and Molecular biology program, University of Arkansas, Fayetteville, Arkansas, United States.

Corticotropic releasing hormone (CRH) neurons located in the nucleus of hippocampal commissure (NHPc) have been proposed to be involved in the avian neuroendocrine regulation of stress and appear to respond prior to CRH neurons in the hypothalamic paraventricular nucleus (PVN). The response, however, has been documented solely from applying one type stressor, food deprivation (FD). We therefore wanted to test whether the response of the NHPc was a stressor specific. Additionally, since the response of the NHPc was rapid and short-lived, was it regulated differentially from the PVN? We therefore applied a psychogenic stressor, immobilization, to determine gene expression of CRH and a panel of stress-related genes in the NHPc, PVN, and anterior pituitary (APit) using RT-PCR and assayed the final stress product, plasma corticosterone (CORT) utilizing RIA. Furthermore, brain derived neurotrophic factor (BDNF) and glucocorticoid receptor (GR) were examined regarding their positive/negative roles in the regulation of CRH neurons. Chicks were subjected to one of the following immobilization treatments when birds were 5 weeks of age: controls (0m), 15m, 30m, 60m, 90m, and 120m. Each group was composed of 12 birds. At each time point, brains were sampled, frozen, and cryosectioned. One-way ANOVA, followed by mean separation among the six treatment groups, was conducted for each gene using a significance level of p<0.05. Data showed that rapid activation of CRH mRNA in the NHPc occurred and preceded a slower gene activation in the PVN resulting in subsequent upregulation of proopiomelanocortin (POMC) transcripts in the APit associated with significant increases in plasma CORT concentrations. Results suggested BDNF’s role in negative feedback observed between CRH and CRHR1 in the NHPc and positive feedback between CRH and CRHR1 as well as AVT and V1aR in the PVN. The GR mRNA expression and protein levels revealed that CRH neurons in the NHPc are regulated by CORT before those in the PVN. In the APit, V1bR activation appeared responsible for sustaining POMC gene expression for continued CORT release when stress persists. Overall, data suggest that the NHPc functions as part of the traditional HPA axis of birds and perhaps a comparable extra-
hypothalamic structure occurs in mammals and other vertebrates.

**Key Words:** Immobilization stress, corticotropin releasing hormone receptors, paraventricular nucleus, glucocorticoid receptors, V1b receptor

**290 Plasma metabolomics and muscle transcriptomics show potential involvement of vascularity issue for increased P. major mixed muscle degradation rate and myodegeneration in myopathy broilers.** Pramir Maharjan¹, Jordan Weil¹, Antonio Beitia¹, Katie Hilton¹, Nawin Suesuttajit¹, Cole Umberson¹, Diego Martinez², Justina V. Caldas¹, Casey Owens¹, Craig N. Coon¹, ¹Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, Arkansas, United States, ²Cobb Vantress, Siloam Springs, Arkansas, United States.

A high yielding meat-type broiler strain was reared in 35 floor pens (25 birds/pen) from 0-56d utilizing dietary specs and husbandry guidelines specific to the breed. The objective of the study was to understand Pectoralis major mixed muscle protein turnover, plasma metabolomics and P. major transcriptomics of broilers affected with Woody Breast(WB) myopathy condition in relation to non-myopathy (normal) broilers. Broilers (n=10) were selected at d 21, 28, 35, 42 and 56 and infused with ¹⁵N-phenylalanine (Phe) and utilized as amino acid tracer. Muscle and ileal digesta samples were collected. ¹⁵N-Phe enrichment and 3-methylhistidien quantitation were performed using mass spectrometry. Fractional synthesis rate (FSR) and fractional degradation rate (FDR) were measured. At d 42, broilers were sampled for histomorphology, body composition (BC) and whole-body heat production (HP) assessments, plasma metabolomics and P. major transcriptomics after separating the sampled birds as WB myopathy (WB score > 2) and normal (WB < 1) (n= 5 each group). Data were analyzed using one-way ANOVA using t-test. Results showed that mixed muscle FSR and FDR decreased (P< 0.05) as broiler aged. Combined analysis of d 42 and d 56 showed higher mixed muscle FDR (%/D) for myopathy broiler (12.88) compared to non-myopathy (10.58) followed by greater degree of myodegeneration occurring in myopathy broiler as shown by histomicrograph results. Fasting HP (P < 0.05) was higher for non-myopathy broiler which was evidenced by higher body protein content given by BC results. Differentially expressed significant metabolites (P < 0.05) identified between myopathy and non-myopathy groups in plasma were homocysteine, cyclic GMP, trimethylamine N-oxide (TMAO), tyramine, carnitine and acetyl carnitine, which were all associated to cardiovascular system. Transcriptomics results showed 50 genes were differentially expressed (P < 0.01) of which also included genes related to cell membrane integrity, vasodilation and blood pressure regulation. Findings suggest that WB myopathy affected broiler could have compromised blood supply in P. major leading to localized ischemia followed by myodegeneration.

**Key Words:** woody breast myopathy, mixed muscle degradation, myodegeneration, plasma metabolite expression
Processing and Products

291 Lipid and protein oxidation of eggs from hens at different ages stored in different environmental conditions. Sarah Maria P. Camargo1, Deibity A. Cordeiro1, Frederico L. da Silva2, Karolyrne M. Nascimento2, Vitor R. Mangerotti2, Letícia V. Chagas2, Juliana M. Fonseca2, Itallo d. Faria1, Aline M. Racanici2, Jose H. Stringhini2,3, 4Zootecnia, Universidade Federal de Goias, Goiania, Goias, Brazil, 5Faculdade de Agronomia e Veterinaria, Universidade de Brasilia, Brasilia, Distrito Federal, Brazil, 6Researchership Holder, CNPq, Goiania, Goias, Brazil.

The goal of this study was to evaluate the effects of storage conditions, hen age and storage period on internal quality of commercial hen eggs. 288 commercial eggs from Hy-line hens at different ages (31, 62 and 88 weeks) were stored for 30 days under refrigeration with an average temperature of 8.05°C or at room temperature with an average temperature of 27.55°C. The experiment was conducted in a completely randomized design in 3x2x2 factorial arrangement (age, environmental condition and storage time) totaling 12 treatments. At the 1st and 30th day of storage, 36 lyophilized samples of each treatment were analyzed in triplicate for lipid oxidation of the yolk (TBARS, μmol/kg) and protein oxidation of albumen (Thiol, nmol/kg). For statistical analysis, a dispersion graph of the data over time was utilized. Higher TBARS values were found on eggs at room temperature at 30 days of storage when compared to those stored in the refrigerator. Considering the hen age, TBARS values were higher for egg yolk from 88-week-old hens on day one (fresh eggs), showing higher levels of lipid oxidation for hens in advanced ages. As the eggs were stored, egg yolk from hens of 31 weeks increased TBARS values vary rapidly, when compared to yolk of hens with 62 and 88 weeks. For Thiols, an increase of protein oxidation of eggs stored at room temperature was noted, whereas the albumen of eggs stored in refrigerator showed a significant increase in Thiol values. When the hen age was considered, an increase on protein oxidation of the eggs from hens of 88 weeks was noted as a decrease in Thiol values. Therefore, high temperatures can accelerate the oxidation process of lipids and proteins of eggs over time and the refrigerator storage prevents the denaturation process of proteins contained in albumen, as well as yolk rancification. In this study, the advanced hen age influenced the internal quality of the eggs, as these eggs showed lower antioxidant activity in the albumen and lower selectivity of yolk membrane. This effect must be taken in consideration on extending of laying hens' life in egg production systems.

Key Words: hen age, preservation, oxidation, yolk, albumen

292 Egg quality in hens housed in two different production systems, conventional cage and cage-free: a commercial study. Roy R. Hernandez1, 2, Edgar O. Oviedo-Rondon1, Ramiro D. Franco2, Edgar R. Aya2, Iang Schronitgen R. Barragán1, 3, 4Poultry Research Group, University of Tolima, Ibagué, Tolima, Colombia, 5Technical Department, Nutriavicola S.A, Cali, Valledel Cauca, Colombia, 6Immunobiology and pathogenesis research group, University of Tolima, Ibagué, Tolima, Colombia, 7Prestige Poultry Science, North Carolina State University, Raleigh, North Carolina, United States.

Egg consumers worldwide have increased their concern about laying hens welfare and its impact on egg quality. This study aimed to compare the egg quality parameters under the conventional cage and cage-free production system in the tropics. The study was carried out on a commercial farm using Hy-Line Brown pullets, reared in cages under the same conditions for the first 15 wk of age. At 16-wk, hens were distributed into two different production systems in the same farm; conventional cage (CC) and cage-free (CF). A CC (40 x 45 cm/four hens) with a stocking density of 450 cm²/hen, for a total population of 45,000 hens, approximately and 180 cages were sampled. The CF had nine hens/m² with a stocking density of 1,111 cm² per hen and a total population of 14,500 hens. In the CC system, 15 replicates with 12 cages each, evaluating location within the house, were assessed and in the CF system, 15 rooms with 1,000 hens/room approximately. Hens in both systems were fed the same diet for each phase, but feed intake varied slightly. Egg samples were collected every six weeks, from 22 to 82 wk of age. A total of 3,960 eggs were analyzed in 11 sampling times. Egg quality was analyzed using a DET6000. Egg weight, albumen height, yolk color, eggshell thickness, eggshell strength were determined, and Haugh units were calculated. Additionally, screening for Salmonella spp. status was conducted at 22 and 82 wk, using environmental and egg samples. For environmental samples, two boot swabs and two surface swabs were collected in each production system. Furthermore, three eggs were pooled for each one of the 15 replicates per production system, each sampling time, and their eggshells and content were analyzed separately using bacteriological culture. Data was analyzed in a completely randomized block design with repeated measures and mean separation by Student’s t-test using GraphPad Prism 6 software. Albumen height (P<0.001), yolk pigmentation (P<0.001), and Haugh units (P<0.001) were higher in CF system compared with CC between 38 until 69 wk of age. In the same way, eggs were heavier at 63 and 82 wk of age (P<0.05) in CF compared to CC. However, from 57 to 82 wk, eggs from CC had better eggshell strength. Salmonella spp. was not isolated from environmental and egg samples after the start of laying and earlier flock depopulation at 82wk. The results suggested that the production system can affect internal and external egg quality parameters, possibly due to different stressor factors such as behavior restriction or environmental conditions. Also, in this study, Salmonella status was not affected by the production system, indicating
the presence of this bacteria may be related to failures in biosecurity in any system.

Key Words: egg-quality, welfare, cage-free, haugh-units, salmonella

293 A comparison between slow and fast-growing strains of broiler chickens on carcass traits and incidence of wooden breast and white striping. Midian Nascimento dos Santos1,2,*, Daniel Rothschild1, Tina Widowski1, Elijah Kiarie1, Ira Mandell1, Michelle Guerin1, Stephanie Torrey1, 1Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, 2Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada.

Wooden breast (WB) and white striping (WS) are myopathies observed in conventional broiler chickens. Selection for slower growth has been suggested to reduce these disorders. This study compared carcass traits and the incidence of WB and WS among 2 conventional (CO) and 9 slow-growing (SL) broiler strains. Using a randomized incomplete block design, 2,552 mixed-sex birds were allocated to 58 pens (44 birds/pen; 30 kg/m²) over 3 production cycles with 4-6 pens/strain. The strains differed in ADG from 44-69 g/d, with ADG=65 g/d for strains C (ADG0-48=65 g/d) and B (ADG0-48=69 g/d), ADG=50-60 g/d for strains H (ADG0-62=52 g/d), E (ADG0-48=53 g/d), F (ADG0-62=56 g/d), G (ADG0-62=56 g/d), M (ADG0-62=56 g/d), and I (ADG0-62=57 g/d), and ADG=50 g/d for strains N (ADG0-62=44 g/d), D (ADG0-62=45 g/d), and J (ADG0-62=48 g/d). Birds had ad libitum access to the same three-phase diet. From each pen, 8 birds (4 males, 4 females) of CO (B, C) and SL (D-N) strains were processed at 48 and 62 days, respectively, based on the final target weight (BWCO=3.24±0.14 kg; BWSL=3.18±0.30 kg). The carcass, breast, drumstick, thigh, and wing weight were measured, and breasts were assessed visually and by palpation for the presence of WB and WS. Data were analyzed using Proc Glimmix in SAS 9.4. With strain and sex as fixed effects, and block and production cycle as random effects, Pearson correlation analysis was conducted to study relationships among WB, WS, and carcass traits. Strain D had a similar carcass weight to strains J and N, yet was lighter than other strains (P<0.01). Higher thigh and drumstick yields were observed in strain D compared to strains B, C, and F (P<0.01). Strain F had similar carcass and breast yield to strains B and C, yet greater than all other strains (P<0.01). Strains C and F showed a higher incidence of WB (P<0.01) and WS (P<0.01) than the other SL strains. Strain B tended to have a higher incidence of WB than strains G (P=0.07) and H (P=0.06) and had a higher incidence of this disorder than the other SL strains (P<0.05). There was no strain by sex interaction on any trait evaluated (P>0.05); males had a higher incidence of WB (P=0.034) and WS (P=0.01) than females. When carcass weight was included as a covariate, sex differences disappeared while strain differences remained, with strains C and F having a similar incidence of WB and WS to strain B, but higher than other SL strains (P<0.01). Both WB and WS were positively correlated with carcass and breast yields but negatively correlated with thigh, drumstick, and wing yields. The incidences of WB and WS observed in both SL and CO strains indicate these myopathies are more related to breast yield rather than growth rate.

Key Words: Broiler chickens, Myopathy, Growth rate, Genotype, Slow-growth

294 Effect of freezing condition and wooden breast severity on cooked chicken quality. Caroline R. Gregg1,*, Dawn Koltes2, Rodrigo Tarte2, 1Iowa State University, Ames, Iowa, United States, 2Animal Science, Iowa State University, Ames, Iowa, United States.

The occurrence of wooden breast (WB) myopathy in broiler chickens is increasing. WB is economically detrimental to the broiler industry since it reduces consumer appeal. Past literature has shown that freezing conditions can influence the quality of a food product. Air blast freezing is used by commercial meat processors to rapidly freeze a product. In comparison, standard household freezers freeze at a slower rate, which results in larger ice crystals within a product. This can damage the cells of a meat product and reduce quality. This study aims to examine the combined effects of WB severity and freezing condition on the instrumental texture of chicken breast fillets. Whole breast fillets were purchased in bulk from a local grocery store and scored for WB severity: 0 (normal), 1 (mild), and 2 (moderate). Six whole breasts per WB severity group per replicate (n=3) were weighed, split into 2 fillets, and each half was placed in either a household freezer (-18°C, HH) or a blast freezer (-40°C, BF). Once the BF samples equilibrated to freezer temperature, all fillets were stored in HH for 4 d. Fillets were weighed, cooked from frozen in an oven on a cookie sheet, cooled to room temperature, and weighed to determine freezing loss and cook loss. Texture analysis was performed in triplicate using a TA-45 incisor probe (Stable Micro Systems) on 3 distinct (cranial, middle, and caudal) regions across the fillet. Data were analyzed in SAS with freezing condition, WB severity, and the interaction fit as fixed effects and raw breast fillet weight included as a covariate for any variable. Raw breast fillet weight was altered by WB severity (P=0.04), where moderate was heavier than normal (P=0.03). Freezing loss was not affected by WB severity (P=0.63); however, HH had increased freezing loss compared to BF (P<0.01). Cook loss was altered by WB severity (P=0.04) with greater cook loss in moderate fillets compared to normal fillets (P=0.04). No significant differences were found in texture analysis across any of the 3 regions (P>0.32). The results of this study suggest that texture qualities of WB-affected meat are not influenced by freezing conditions and wooden breast severity.

Key Words: Air Blast, Household Freezer, Incisor Probe, Wooden Breast
Textural and physical properties of breast fillets with myopathies (wooden breast, white striping, and spaghetti meat) in fast-growing broiler chickens in Canada. Chaoyue Wang, Sunoh Che, Christian Fuchs, Shai Barbut, Leonardo Susta, Pathobiology, University of Guelph, Guelph, Ontario, Canada; Food Science, University of Guelph, Guelph, Ontario, Canada. Food Safety and Quality Assurance, Maple Leaf Foods, Mississauga, Quebec, Canada.

Myopathies such as Wooden Breast (WB), and White Striping (WS) are some of the known defects costing the industry millions of dollars. Recently, a new type of myopathy called Spaghetti Meat (SM) start to cause important damage to the industry. Different types of myopathies are not mutually exclusive; therefore, it is pertinent to categorize breast fillets into a combination of myopathies. The aim of our study was to evaluate these parameters in samples (N = 84, WB1 WS1 = 40, WB1 WS1 SM1 = 41, WB2 WS1 = 57, WB2 WS1 SM1 = 69, WB2 WS2 SM1 = 33) gathered from poultry processing plants in Ontario. Each sample was categorized based on the severity and combination of the three myopathies (WB, WS, SM), scored as follows: 0: absent; 1: mild, noticeable upon close inspection; 2: severe, noticeably altered from normal breast). Breast without any myopathy was labeled as normal (N). One-way Anova with Tukey’s multiple comparisons was performed to compare samples’ means. Samples with any combination of myopathies resulted in higher drip loss compared to Normal fillets, while the presence of SM further increased drip loss. Individual results are presented in the order of N, WB1 WS1, WB1 WS1 SM1, WB2 WS1, WB2 WS2 SM1, and WB2 WS2 SM1; numbers followed by different letter are significantly different (P<0.05). The result of drip loss were: 0.71%a, 0.92ac, 1.37bd, 1.11cd, 1.36de, 1.70e. Only severe WB showed significant increase in cooking loss, and the results were: 15.56%a, 16.32ab, 17.04af, 19.96cg, and 19.67dh. Presence of WB increased the pH significantly; results were: 5.74a, 5.79ab, 5.73a, 5.87b, 5.88c, and 5.87b. WB significantly increased the compression force (in Newtons), while SM decreased it. Overall, SM was the most damaging because SM1 was enough to affect compression force and drip loss, whereas only severe WB significantly affected these properties. Lastly, WS has the least effect on the quality of the breast fillets.

Key Words: broiler myopathy, spaghetti meat, texture, white striping, wooden breast

Myowater properties during the first 24 h postmortem and their relationship to final moisture content. Giulia Tasoniero, Hong Zhuang, Brian Bowker, USDA, Agricultural Research Service, Athens, Georgia, United States.

The aim of this study was to provide insight on changes in myowater mobility and distribution in breast fillets within the first 24 h postmortem (PM) and determine their relation to final moisture content. To this purpose, 12 broiler carcasses were collected at 15 min PM. For each carcass, the right breast fillet was deboned immediately (hot deboned) while the left fillet was deboned at 2 h PM after chilling in ice-water slush. The nuclear magnetic resonance (NMR) traits (T21, T22, P21, P22, T2B, P2B) of intact right fillets were assessed at 45 min and 1.25, 2, 3, 4 and 24 h PM. In intact left fillets, NMR traits were measured at 2, 3, 4 and 24 h PM. At 24 h, total moisture content was determined in all fillets according to AOAC Official Method 2008.06. A one-way ANOVA mixed model was performed to evaluate postmortem time as a fixed effect within deboning time with sample ID as random effect. Pearson correlation within deboning time was performed between NMR traits measured at each time and final moisture content. In hot-deboned fillets, the mobility (T) and proportion (P) of the water tightly bound to macromolecules (T2B and P2B), intramyofibrillar (T21 and P21) and extramyofibrillar H2O (T22 and P22) were strongly influenced by postmortem time. Bound water exhibited the highest mobility (P < 0.0001) coupled with the lowest proportion (P = 0.0036) between 2 and 4 h PM. The mobility of the intramyofibrillar water compartment (T21) decreased from 45 min to 2 h PM and then increased from 3 to 24 h PM (P = 0.0002). The proportion of intramyofibrillar water (P21) was at its minimum at 1.25 h and then reached its peak at 4 h PM (1.25 h = 69.8% vs 4 h = 79.2%; P < 0.0001). The mobility of the extramyofibrillar water compartment (T22) was steady between 45 min and 4 h PM and then decreased to reach its minimum at 24 h PM (P < 0.0001) while P22 reached its peak at 1.25 h and then decreased to its minimum at 4 h PM (1.25 h = 29.5% vs 4 h = 20.1%; P < 0.0001). The NMR traits of fillets subjected to post-chill deboning exhibited similar trends. The correlation analysis revealed that the total moisture content at 24 h PM is highly and positively related to T21 (0.78) and P22 (0.76), but negatively related to P21 (-0.75). Specifically, the highest correlation was found between moisture content and T22 measured at 2 and 3 h PM (-0.85). To conclude, this study indicated that changes in myowater distribution and mobility occur in breast fillets within the first 24 h postmortem; it also suggests that the mobility of the intramyofibrillar compartment at 2 and 3 h postmortem is a factor that greatly impacts the final moisture content of meat.

Key Words: chicken breast meat, postmortem time, NMR, total moisture content

A Comparative study on carcass and meat quality traits of three Sri Lankan indigenous chicken strains reared under semi-intensive system. Madushika K. Ranasinghe, D.D. Jayasena, Department of Animal
Carass and meat quality traits of Sri Lankan indigenous chicken strains need to be studied as there is no scientific literature recorded. Hence, the present study was conducted to compare the carass and meat quality traits among three indigenous Sri Lankan chicken strains namely; Village/Non-descriptive (VC), Naked Neck (NN) and Long Legged (LL) chicken. A total of 54 birds at similar age and live weight (1.3 kg – 1.8 kg) from both sex of the three indigenous chicken strains reared under semi-intensive system were purchased and sacrificed according to the commercial guidelines. Live weight, carcass weight and dressing percentage were recorded initially and the whole breast and leg muscle from each bird were used to determine pH, colour, water holding capacity (WHC), cooking loss, proximate composition and fatty acid profile. Data from live weight, carcass weight and dressing percentage were analyzed using one-way ANOVA while data from other parameters were analyzed using two-way ANOVA with General Linear Model in Minitab 17®. Amongst the strains parameters were analyzed using two-way ANOVA with Balchem Corp., New Hampton, New York, United States. Science, Uva Wellassa University, Badulla, Sri Lanka.

During broiler processing co-products such as Wooden Breast (WB) meat and carcass frames (CF) are generated. Low quality and the textural defects of WB meats leads to low acceptance from consumers and may cause significant economic losses. Although WB and CF are overlooked meat products, they are potential resources for development of pet treats. Physical characteristics like color are one of the contributing factors to desirable organoleptic characteristics which are critical to the consumer’s willingness to purchase final products. An experiment was designed to evaluate effects of sodium alginate + encapsulated calcium lactate (PetShure® Structure & Forming Agents, Balchem Corp., New Hampton, NY; ALGIN) additions on color variation (CV) of pet treats made from a wide range of WB and CF proportions over a period of 5 d post-production. CF and score-3 WB fillets were ground to 10 mm. Ground products were mixed to achieve the following 11 ratios of ground CF to WB: 100% CF:0% WB, 90% CF:10% WB, 80% CF:20% WB, 70% CF:30% WB, 60% CF:40% WB, 50% CF:50% WB, 40% CF:60% WB, 30% CF:70% WB, 20% CF:80% WB, 10% CF:90% WB, 0% CF:100% WB. Subsamples of the 11 CF:WB mixtures were then mixed with 3 different dosages of ALGIN (0.5, 1, and 2% sodium alginate mixed with 0.425, 0.85, and 1.7% calcium lactate, respectively) to produce the final batches (n = 33), stuffed into 40-mm sausage casings, and frozen at -20°C for 48 h prior to slicing. Raw product was cut into 10-mm slices and 10 slices from each batch were selected for measurement of L*, a*, b* CV using a Minolta colorimeter on the top cut surface after 1, 3, and 5 d of storage. L* represents lightness of samples, which varies from 0 when black to 100 when white. Value a* represents redness and varies from green to red while b* represents yellowness of samples which varies from blue to yellow. Both a* and b* values vary from -120 to +120. Data were analyzed using GLIMMIX procedure of SAS and means were separated using the PDIFF option at P ≤ 0.05. An interaction was observed among WB:CF combinations and ALGIN inclusion for all L*, a*, b* values (P ≤ 0.0001). Samples with higher WB concentration and ALGIN dosages were lighter on d 1, 3 and 5 (P ≤ 0.0001). Redness decreased as CF increased on d 1, 3 and 5 but with higher ALGIN dose redness slightly increased on d 1, decreased on d 3 and remained constant on d 5 (P ≤ 0.0001). Yellowness increased as ALGIN dose increased in pet treats on d 1, 3 and 5 (P ≤ 0.0001) but decreased as WB increased on d 1, 3 and 5 (P ≤ 0.0001). Adding sodium alginate and encapsulated calcium lactate while increasing Wooden Breast meat proportion increased lightness of pet treats.

Key Words: color variation, broiler chicken processing, Wooden Breast Meat, alginate, pet treat

298 Analysis of color variations over time of raw pet treats made from co-products of broiler chicken processing. Moses E. Chilenje(*)1, Marc R. Presume1, Lindsey F. Spencer1, Catherine M. Odom1, Laura J. Garner1, Amit Morey1, Robert P. Mason2, Eric K. Altom2, Charles W. Starkey1, 1Poultry Science, Auburn University, Auburn, Alabama, United States, 2Animal Nutrition and Health, Balchem Corp., New Hampton, New York, United States.

299 Evaluation of the textural characteristics of pet treats generated from mixtures of broiler processing co-products. Marc R. Presume(*)1, Lindsey F. Spencer1, Catherine M. Odom1, Laura J. Garner1, Amit Morey1, Robert P. Mason2, Eric K. Altom2, Charles W. Starkey1, 1Poultry Science, Auburn University, Auburn, Alabama, United States.
Broiler chicken carcass frames (CF) and Wooden Breast meat (WB) are often undervalued but possess great potential for generating new products. Quality of these meat products is often determined by their organoleptic characteristics, such as texture. The objective of this study was to evaluate textural characteristics (TC) of pet treats generated from mixtures of CF and WB. CF and severely affected WB were ground to 10 mm. Resulting ground products were mixed to achieve the following 11 ratios of CF to WB: 100% CF:0% WB, 90% CF:10% WB, 80% CF:20% WB, 70% CF:30% WB, 60% CF:40% WB, 50% CF:50% WB, 40% CF:60% WB, 30% CF:70% WB, 20% CF:80% WB, 10% CF:90% WB, 0% CF:100% WB. Subsamples of the 11 CF:WB mixtures were then mixed with 3 different dosages of ALGIN (0.5, 1, and 2% sodium alginate + 0.425, 0.85, and 1.7% calcium lactate, respectively) to produce the final batches (n = 33), stuffed into 40-mm sausage casings, and frozen at -20°C for 48 h prior to slicing. Meat products were cut into 10-mm slices for analysis of TC. For raw product, hardness, adhesiveness, resilience, and cohesiveness were measured using 2-cycle compression with a TA-40 cylinder probe. On cooked product, raw samples were dehydrated at 107°C for 2 h and cooled to room temperature, then firmness and toughness were analyzed with a TA-42 blade, while gradient, peak force, and flexibility were measured using a TA-92 3-point bend apparatus. Data were analyzed with the GLIMMIX procedure of SAS and means were separated at P ≤ 0.05. Hardness increased as WB proportion (P ≤ 0.0001) and ALGIN inclusion increased (P ≤ 0.0001). Adhesiveness also increased with increasing WB (P ≤ 0.0001), peaked at 90%WB and decreased as ALGIN increased (P = 0.0068). Resilience of raw samples was inversely proportional to WB (P ≤ 0.0001) and ALGIN (P ≤ 0.0001), whereas samples with higher WB and ALGIN were more cohesive (P ≤ 0.0001). No interaction was observed among WB-CF combination and ALGIN inclusion for thickness of dehydrated samples (P > 0.05). Gradient increased with decreasing CF (P ≤ 0.0001) and increasing ALGIN dosage (P = 0.0065). Peak force values of dehydrated samples were increased as CF (P ≤ 0.0001) and ALGIN decreased (P ≤ 0.0001). As WB (P ≤ 0.0001) and ALGIN increased (P ≤ 0.0001), flexibility increased, peaked at 60% WB and then decreased. Firmness and toughness increased as WB content (P ≤ 0.0001) and ALGIN dosage increased (P ≤ 0.0001), peaked at 80% WB before decreasing. In conclusion, reduced value broiler processing co-products were successfully repurposed to generated pet treats. Value can be added to poultry processing co-products to improve their utility and generate quality high-value products for substantially greater economic returns.

**Key Words:** textural characteristics, broiler chicken processing, Wooden Breast meat, alginate, pet food

**Peracetic Acid Half-Life Rates.** Daniel Sabo*, Stephanie Richter, Marc Zanghi, Georgia Tech Research Institute, Atlanta, Georgia, United States.

The poultry industry relies on peracetic acid (PAA) as an antimicrobial to ensure food safety. This heavy reliance highlights the need to clearly understand PAA chemical decay rates within immersion chillers and factors attributing to decay. Anecdotal evidence report PAA decays rapidly when in residence with high organic carbon loading. Organics are common in the chiller and appear in the form of total suspended solids (TSS), total dissolved solids (TDS), fats, oils, and greases (FOGs). To date, the exact contributors of PAA decay have not been documented. Reports also show that pH effects PAA decay and stability. This research effort aims to determine the exact half-life of PAA. Experiments were conducted using simulated chiller media with and without pH adjustments. Simulated chiller water mimicked concentrations similar in the main chiller water: TSS, TDS, COD, FOGs, and pH ranges. Whole carcasses (WOGs) were added into water at ratios used within industry. Three industry-grade PAA solutions, ranging from 15% to 22% peracetic acid, were tested. PAA concentration was measured using two colorimetric adsorption device kits and two colorimetric titration kits. For all tests, initial PAA concentrations targeted 100 ppm. PAA was added to test water and mixed for sixty seconds before ppm was calculated. This measurement represented t=0. All studies measured PAA concentration at six time points (0, 5, 15, 30, 60, 90, 120 minutes) for three pH ranges (4.5, 8.2, and 10). PAA decomposition in potable water acted as a control group for decay rate comparison. Results from t=5 minutes for organic loaded water 82% (pH10), 35% (pH 8.2) and 79% (pH 4.5%) PAA ppm remained from t=0 (P-value ≤0.05). In the presence of organic carbon, with no pH adjustments, PAA half-life ranged between 120 to 200 minutes. Tests with pH adjusted to 4.2 or 10 with organics, PAA half-lives measured approximately 15.09 to 17.19 minutes respectively. These results indicate a compounding effect of pH and organic loading on PAA concentration and stability within a chiller. PAA may not be at target concentrations as carbon content within chillers increases throughout a processing shift.

**Key Words:** PAA, concentration, organic load, immersion chilling, peracetic acid

**301 Alternative Approaches to Broiler Transport: On-Farm Processing System (FPaT).** Alex V. Samoylov*, Georgia Tech Research Institute ATAS, Georgia Tech, Atlanta, Georgia, United States.

The humane treatment of broilers during processing is a priority for the poultry industry. Pre-slaughter routines such as broiler catching and transport are the most stressful stage of the broiler production process, with known physiological and behavioral effects. As part of this process, broilers are manually captured, crated, and loaded onto trailers for transport from the grow-out farm to the processing facility.
During this period, they experience heat and environmental stress, bruising, and injury. These welfare issues are endemic to the current process and not easily addressed through incremental innovation. It is time to reimagine how broiler transport works and the proposed On-Farm Processing and Transport System (FPaT) has a chance to do that. The goal is to develop an alternative broiler chicken transporting methods that dramatically improve animal well-being by eliminating live transport and transporting carcasses instead. The envisioned mobile FPaT system improves the process by stunning, slaughtering, and shackling the broilers at the farm. A separate unit then transports the shackled carcasses to a poultry processing plant. The hypothesis is that the FPaT approach will dramatically improve bird well-being by reducing live handling, eliminating live transport, eliminating DOAs, reducing water and energy consumption, reducing labor, and providing better working conditions for catching crews. For an initial assessment of the operability of the FPaT system teams from Georgia Tech Research Institute, the University of Georgia, and USDA processed broilers using prototype shackle transport system and tested for carcass damage during the trip, pickability, and meat quality. Four groups, one control and three treatment groups, were tested. The control group consisted of 10 broilers that were processed using traditional methods including water-bath electrical stunning, and three treatment groups were processed using FPaT system. The three treatment groups processed through FPaT system consisted of 10 broilers each using three different stunning methods: CAS stunning, LAPS, and water-bath electrical stunning. All carcasses were visually examined for physical damage and did not show differences between control and treatment groups. Carcasses also had a visual inspection of the de-feathering process. The results showed slightly more feathers retained by carcasses of the treatment group but not enough to cause concerns. Also, the team collected and examined traditional meat quality parameters such as pH, cook loss, lean color, and texture and did not observe large differences. This was a preliminary trial and it will require more repetitions to obtain statistically significant results.

Key Words: Transport, CAS, LAPS, Well-being, Broiler

302 Predicting Anatomical Structures to Improve Automated Deboning. Louise L. Zhuang1*, Wayne Daley, Food Processing Technology Division, Georgia Tech Research Institute, Atlanta, Georgia, United States.

Due to rising market demand for deboned poultry parts, high rates of worker turnover in poultry plants, and decreasing cost of robotic systems, automated deboning offers a viable solution for matching global poultry needs. A key element of automation is the development of trajectory determination methods that provide flexibility in accommodating natural product variability. Bird by bird knowledge of the location of key anatomical structures would facilitate the optimization of cutting trajectory paths. However, sensing systems such as X-ray do not resolve soft tissues and other sensing modalities like CT or MRI are slower and costlier. The main objective of this work was to create a model to rapidly and accurately predict anatomical structures, focusing on the key ligaments about the shoulder joint that need to be severed when performing a wing cut. The development of a prediction model involved identifying the major ligaments (medial and lateral) and their endpoints from chicken MRI scans, followed by identifying the corresponding endpoint locations on 3D chicken meshes created from CT scans in an interactive virtual reality (VR) environment. Training and testing of the anatomical model then occurred using the VR system. Points were placed for model training on the endpoints for each of the medial and lateral ligaments on both sides of an initial set of 14 large chicken skeletons with an average underwing span of 82.5 mm and 14 small chicken skeletons with an average span of 66.9 mm. A least-squares fit was then performed on the endpoint position data generated by using a reference frame defined by points on each chicken’s body mesh in order to create an anatomical prediction model. To analyze the accuracy of the ligament endpoints determined from the model, the distances between computed endpoint and actual endpoint locations were compared for 27 large and small test birds. The mean difference in distance between calculated and actual endpoint locations for large birds was 4.42 mm, with a 2.12 mm standard deviation; the mean difference in distance for small birds was 3.53 mm, with a 1.60 mm standard deviation. Some bias was evident in the coordinate errors for the y (sagittal) and z (vertical) components, which merits further investigation. Based on these results, predicting key anatomical features using a least-squares model given input reference points on the surface of the body appears promising. With additional data, a more refined model to predict within a 5-mm accuracy will allow for the design of automated deboning and other handling devices to make automation of operations in second and further processing of poultry more tractable.

Key Words: robotics, automation, modeling, deboning, virtual reality

303 Virtual Reality Robotic Cutting Design Framework. Sim Harbert*, Konrad Ahlin, Walker Byrnes, Nadun Ranawaka, Louise Zhuang, Stephanie Richter, Food Processing Technology Division, Georgia Tech Research Institute, Atlanta, Georgia, United States.

As research continues to improve dynamic cutting robots based on various sizes and types of birds, an automated technique for optimizing the cutting trajectories is useful. This project creates a modular system for quickly taking cutting trajectories from a virtual reality testing environment to a physical robotic testing environment. This research creates a tool for designing and editing cutting trajectories for performing bird deboning cuts by allowing for the creation of the cutting trajectories using a virtual reality (VR) app that can then be quickly translated to motion commands for a physical robot to test on real birds. By using the VR environment to visualize and test the
cutting trajectories on virtual 3D models of the birds including bones and skin, the trajectories can be modified and optimized without needing to test on real birds. The ability to translate those trajectories quickly to the physical robotic environment allows for easier validation of the created cutting trajectories. The VR app for visualizing and modifying the cutting trajectories is created using the Unity gaming system and tested on the HTC Vive VR headset. This VR environment is connected over the network to a module that acts as a database for the cutting trajectories that are created. The physical robot consists of a UR5 robotic arm controlled by a computer running the Robotic Operating System (ROS). This robot controller requests the trajectories from the trajectory database module and performs the cutting trajectory motions. Basic analysis of the cutting trajectories in the VR environment is performed by reporting intersections of the virtual knife blade with the 3D bird skeletal model. Further analysis of the cuts performed by the physical robot will be generated. The entire modular system allows for real-time testing of the cutting trajectories generated in the VR environment with the physical robot on real birds. This means having a system where quick iterations of editing the trajectories and testing with the robot are possible.

**Key Words:** virtual reality, deboning, robotics, cutting, 3D modeling

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304  Differences in bioelectrical properties of breast meat affected with woody breast, white striping and spaghetti meat between three fast-growing big broiler flocks. Jaroslav Valenta12, Aftab Siddiqui1, Laura J. Garner1, Amit Morey1. 1Poultry Science, Auburn University, Auburn, Alabama, United States, 2Animal Science, Czech University of Life Sciences, Prague, Czechia.

Breast meat myopathies such as woody breast (WB), white striping (WS) and spaghetti meat (SM) affect meat quality which must be detected and removed from the processing lines. Hand-palpation or manual methods are not reliable and there is a need to develop rapid and accurate detection technologies. Bioelectrical impedance analysis (BIA) was used to study the differences in bioelectrical properties of breast meat affected with WB, WS and SM in three flocks of broilers. Random sample of 100 breast fillets/flock was taken from the deboning lines, weighed individually (avg wt. 477.75 ± 65.08), hand-palpated for WB score (0: Normal, 1: Mild, 2: Moderate, 3: Severe), visually scored for WS (0: Normal, 1: Mild, 2: Moderate, 3: Severe) and pinched in the cranial region to detect absence (0)/presence (1) of SM. The fillets were then subjected to BIA (resistance Rs and reactance Rx). Experiment was repeated over 3 different fast-growing big broilers. Data was analyzed using ANOVA with Tukey HSD to detect differences between severity scores of each myopathy and between each flock. Average WB occurrence in the three flocks was 0 - 28%, 1 - 21.3%, 2 - 27.3% and 3 – 23.3% while 25% of breast meat from the three flocks had presence of spaghetti meat. Severe WS was observed in 1.7% of the fillets analyzed from the three flocks. Data indicated that there were overall significant differences (p<0.05) in the bioelectrical properties (Rs and Rx) different between WB and SM severity levels while no significant differences (p>0.05) were observed in WS scores. Compared to WB-3, Rs for WB-0 was the significantly higher (p<0.05) for flock 1 (71.99 Ohm) and flock 2 (70.47 Ohm). WB-0 fillets from flock 3 had the highest (p<0.05) Rs (78.02 Ohm) compared to the other two flocks. Rs were significantly higher in SM-0 in flock 1 (71.44 Ohm) and flock 2 (67.92 Ohm) compared to SM-1 (67.98 and 66.37 Ohm, respectively). Rx of SM-0 from flock 2 (31.13 Ohm) was significantly lower (p<0.05) than that of flock 1 (37.96 Ohm) and flock 3 (38.32 Ohm). Broiler breast meat bioelectrical properties can be used to differentiate between different WB and SM myopathy severity levels. The experiments indicated that the bioelectrical properties differ among different flocks of chicken. So, processors should collect BIA data from different flocks prior to setting the bioelectrical parameters for detecting myopathies.

**Key Words:** Bioelectrical impedance analysis, woody breast, spaghetti meat, resistance, reactance
The 2050 challenge is real: throughout our lifetime, the global human population will triple without a concurrent increase of natural resources to produce more food. As a result, agriculture will have to become much more efficient worldwide and engage in an efficient path similar to the one it has traveled down in U.S. livestock and poultry production in recent decades. It will take collaboration at all levels of the food system and sound science to meet this challenge.

Key Words: poultry production, natural resources, 2050 challenge
Managing the impact of enteric diseases such as necrotic enteritis and coccidiosis on broiler health and performance is a constant hurdle for integrated poultry companies. Because coccidiosis presents challenges both directly and as a contributing factor to the onset of necrotic enteritis, adoption of nutritional strategies to aid the bird in coping with this disease, particularly in the subclinical form in which it often occurs in the field, is a key part of successful poultry production in the absence of in-feed antibiotics or ionophores. As such, we have studied the nutritional implications of models based on repeated infections of multiple Eimeria species for broilers in battery cages as well as in floor-reared broilers given the opportunity to cycle oocysts naturally. Compared with assessing impacts at peak damage following a single challenge, these models better mimic the magnitude and duration of intestinal damage and inflammation experienced by birds in the field. This has facilitated a better understanding of the effects of coccidiosis on nutrient utilization, as well as potential intervention strategies, throughout all stages of infection and recovery.

**Key Words:** coccidiosis, Eimeria, nutrition

Poultry meat and egg production is an efficient and low-cost source of protein for human consumption. These products remain economical for several reasons including but not limited to advances in genetics, nutrition, bird health and management. Management is significant factor in poultry reaching their full genetic potential in terms of production. Environmental control one part of management which focuses on reducing bird stress by providing optimum conditions for the birds in rearing and production housing. Environmental factors that can affect bird performance and subsequent data collected from pen and field trials include temperature, relative humidity, air velocity, and light program. This presentation is going to review the importance of monitoring these variables and water consumption when it comes to field trials. Too often do people present data that has points that seem irregular and cannot be explained. Often time the reason these values cannot be discussed is that the data were not recorded. I have never heard anyone complain about having too much data, but I have often times heard regret or frustration over not having a certain measurement. Environmental data that should be recorded regardless of the study include room temperature, room relative humidity, and water usage. In addition, the controller setup should be recorded in the project notes it is known when fans, evaporative cooling systems, lights, or heat is operated.

**Key Words:** House environment, Monitoring house environment, Water consumption

The ultimate goal, when conducting an animal trial either in a research facility or in the field, is to correctly understand the effect of treatments (inputs) on animal responses (outputs). A goal is to minimize, as much as possible, the variation between experimental units which isn’t contributed by the experimental treatments. For example, starting a trial with poor quality chicks or having starting weights which are statistically different across treatments, or between the experimental units (pen, farm, barn, etc.), adds unnecessary variation and could result in a biased result. Even though it can be difficult and time consuming to achieve, having equal starting weights between experimental units should also be a goal. Ideally, only one gender should be used within a study, not only due to significant differences which exist in performance, but it also adds to the difficulty in correctly adjusting for mortality across treatments and experimental units. In addition, a mixed sexed trial typically has more variation making it more difficult to detect a significant difference between treatments. If gender is of interest, there are methods to properly design and include that information in the statistical analysis. It is very important to assure the experimental process is both precise and accurate and that a sufficient sample size, selected from the population, is used and properly measured to be able to detect a ‘true’ significant difference between treatments. Using an adequate number of both birds per experimental unit and treatment replicates is vital in having a successful trial outcome. Blocking of treatments within the barn or farm, is a design technique which can be used to statistically account for some of the inherent variation within a system. Before staring a trial, clear objectives and a stated hypothesis should be set. The significance level, also known as the Type I error or a, is the probability level set for rejecting the null hypothesis when it’s true. In order to decide which significant level to use (0.1, 0.05 or 0.01), one should consider the consequences of Type I error. In field trial, an a level of 0.05 (there is only a 1 in 20 chance of rejecting the null hypothesis when it’s true or false positive) are not easy to achieve. Perhaps an a level of 0.1, or there is a 2 in 20 chance of finding significantly different when in reality there is no different, could save the company money. Thus, it should be fully understood that any reasonably acceptable a level for significance can be set in practical situations, especially when economics are applied to the data.
Key Words: Statistics, field research, trial design, experimental design, statistical analysis

309S  A field veterinarian’s perspective: What does all this data mean in decision making? Elizabeth Dale*, Pilgrim’s, Athens, Georgia, United States.

No description provided.

Key Words: N/A


Data evaluation and interpretation is a frequent activity for poultry industry professionals. While evaluating data, it is important to determine whether the conditions of the trials are applicable for implementing an idea or product in a specific production system. In some instances, academic trial design and conditions may not have direct implications in practical situations. For a field nutritionist, the genetics, raw materials, environment, coccidiosis control programs, and stocking density, among others, should be considered when determining whether benefits proven in research trials will translate into an advantage in the field. After an idea or product has been demonstrated to be beneficial in research trials, nutritionists often evaluate ideas or products in an environment that better emulates their production system. This may include internal pen trials, paired house trialing, or complex evaluations. Nutritionists are challenged to defend their decisions and field trials are a common way to bridge the research gap.

Key Words: nutrition, research, field trials, product evaluation
Informal Nutrition Symposium: Ingredient Quality Assessment for Old and New Ingredients

311S Feed evaluation: NIR technologies, quality system and utilization. Alessandro Stercoli*, Cargill, Fiorenzuola d’Arda, Piacenza, Italy.

The goal of any feed formulation process is to respond appropriately to wildly fluctuating feed ingredient and livestock markets and control variation in nutrient content of feedstuffs in order to deliver final products that are: quality-proven, safe, healthy, sustainable and cost effective. Animal performance is not about the feedstuffs the animal is fed; it’s about the nutrients delivered, associated to an healthy animal. The entire manufacturing process, starting from the nature of the ingredients, sampling, analyzing, and processing is subject to errors that introduce variability. From a measurement perspective the biggest focus is typically on having analytical methods that are as accurate and precise as possible (sometimes not possible!). The aim of this speech is to understand how a today largely known technology like NIR, can benefit this system if used for its potential (understanding weaknesses and strengths) and what it takes to be maintained effective and productive.

Key Words: NIR, quality system, nutrients

312S Using near infrared spectroscopy results in commercial diet formulation. Peter V. Chrystal*1,2, PRF, University of Sydney, Pendle Hill, New South Wales, Australia, 1Baiada Poultry, Pendle Hill, New South Wales, Australia.

Access to data in modern poultry production is unprecedented and this applies equally to information on feed and feed ingredients. Laboratory wet chemistry analysis, using the latest equipment, is more accurate than ever before whilst the successful development of near infrared spectroscopy (NIRS) has resulted in an inexpensive manner of analysing a wide range of nutrients in feeds and feed ingredients. This presentation considers the use of enormous amounts of data generated through NIRS analyses on raw materials and suggests methods of applying this data successfully in feed formulation, at a commercial level. Furthermore, the importance of finished feed in relation to raw material analyses is addressed.

Key Words: Near infrared spectroscopy, Feed ingredients, Nutrients, Feed formulation

313S Applying feed evaluation results – accurate delivery of formulated nutrients. Mike Blair*, United Animal Health Inc., Woodstock, Georgia, United States.

Once NIRS data for feed ingredients have been generated, implementation into feed formulation and the making of the feed in the feed mill can be challenging, yet this is of critical importance. Validation of NIRS results with wet chemistry is an important step. For implementing a new program based on NIRS results, 20 to 30% of the samples analyzed by NIRS should be submitted to an approved laboratory for validation and thereafter an ongoing validation of 10 to 20% should be implemented. For an efficient NIRS program, valid nutrient data for key nutrients set up in the feed formulation system (those with minimum and/or maximum levels for each feed) should be the focus, as the data generated using NIRS is overwhelming. Accurate transfer of NIRS data into formulation is the first critical step. Taking advantage of this in the making of the feeds in the feed mill is the next critical step. Practical application in the feed mill will be addressed e.g. real time formulation versus formulating on historical data, limitation of ingredient bins and tailoring the data to fit scaling limitations will be presented as “food for thought” for nutritionists.

Key Words: NIRS, Feed Ingredients, Feed Formulation, Feed Milling

314S Strategies to evaluate novel ingredients for poultry diets. Rex W. Newkirk*, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

The feed industry provides a valuable service to society by utilizing by-products to create high-quality protein in the form of meat for humans. However, before incorporation into diets, new ingredients must be evaluated to determine both their safety and efficacy for the target species. In this presentation, Dr. Newkirk will focus on the process to determine the safety and efficacy of novel ingredients as well as describe the approval process required by CFIA in Canada and AFFCO in the United States. In addition to these legal requirements, the feed industry also requires detailed nutrient profiles, recommended quality control parameters and information on the handling, storage and processing of the ingredient. This presentation will discuss two recent examples to demonstrate the process. Dr. Newkirk will explain the process The Canadian Feed Research Center recently used to evaluate and register Camelina meal. He will also discuss the current approach that the Hemp industry is using to obtain approval for their ingredients and introduce them to the market.

Key Words: Novel Ingredients, Evaluation, Registration


As global demand for protein rises, the development and commercialization of new protein sources will be critical to maintain protein inputs for animals and people. Sustainable production systems are also a major consideration in the development of new proteins. Insect-based products are of
considerable interest for the very high yield potential, with lower land/water/feed inputs (species dependent) and also for the tremendous potential to supply essential nutrients including amino acids, fatty acids, and many micronutrients. Additional benefits of insect derived ingredients may include the prebiotic effects of chitin, as well as anti-microbial activities of various protein and lipid fractions. While much research has focused on insects for aquaculture diets, there is considerable work in poultry and swine, and more recent work in pet nutrition demonstrating the potential value of insects in applied feeding programs. An overview of the current knowledge of insect derived ingredients in animal feeds, with an emphasis on poultry nutrition, will be presented.

**Key Words:** insect, protein, alternative ingredient
**Educating the Next Generation of Poultry Scientists on Bioinformatics**

**316S  Standardizing microbiome analytics for the poultry grower.** Kristina M. Fey*, University of Arkansas, Fayetteville, Arkansas, United States.

Pre-harvest microbiota research is increasingly common; however, the use of the research for integrators has yet to be firmly established. Research is all over the place and lacks the necessary standardization to enable producers to truly apply the data. Additionally, while popular, compositional data may not necessarily be the most important component of research as the biology behind the microbiota is enigmatic. Instead, other metrics may be more important to understand how the microbiota responds to stress and protects the host. Therefore, the Animal and Agriculture Microbiome Consortia was founded in order to bring about that standardization and initiate that discussion to enable the producer to better understand the data and the application thereof. By pushing for standardization of bioinformatics, data acquisition, and research design, understanding microbiota data and the application thereof will become more accessible.

**Key Words:** Preharvest control, standardization, microbiota, bioinformatics

**317S  Delivery of bioinformatics to poultry processing.** Steven C. Ricke*, University of Arkansas, Fayetteville, Arkansas, United States.

Poultry processing is undergoing changes both in operations as well as microbial methodologies. Traditionally microbial data has been gathered in a series of culturing methods using liquid media and plating for isolation and enumeration. Both foodborne pathogens and non-pathogenic bacterial populations are estimated to assess food safety risks as well as potential for spoilage. Bacterial loads from carcasses are important for assessing processing control and the effectiveness of antimicrobial applications. However, these culture-based approaches may only provide part of the microbial ecology landscape associated with chicken carcasses and the subsequent changes that occur in these populations during processing. Newer molecular-based approaches such as 16S sequencing of the microbiota offers a means to retrieve a more comprehensive microbial compositional profile. However, such approaches also result in large data sets which must be analyzed and interpreted. As more data is generated this will require not only bioinformatic programs to process the data but appropriate cyber security to protect the processed data.

**Key Words:** Poultry processing, microbiota, bioinformatics

**318S  Communicating bioinformatics to small poultry producers.** Michael J. Rothrock*, USDA-ARS-USNPRC, Athens, Georgia, United States.

The use of "omics" has become more common in a variety of aspects of poultry production, from breeding to management to bird health to food safety and everywhere in between. While the conventional poultry industry has become more exposed to the power and utility of "omics", smaller poultry producers typically do not have this same level of exposure. Since smaller, non-conventional poultry production is a growing portion of the overall poultry market, it is important that they also have education and access to these research tools and the data produced by these tools. While these small producers are dedicated and knowledgeable farmers, their knowledge of these newer technologies is limited at best, and it is our job as researchers to communicate the importance of these "omic" tools and how the resultant data can improve a variety of different aspects of their operations. I will discuss the ways to effectively communicate complex microbiota and whole genome sequence data to small producers into meaningful and applicable information that they can utilize to inform beneficial management changes.

**Key Words:** microbiome, small producers, outreach

**319S  Communicating complex data sets to poultry nutritionists.** Joshua Jendza*, BASF Corporation, Basking Ridge, New Jersey, United States.

The worlds of animal nutrition and animal health have historically been viewed as related but still distinctly separate fields. As our understanding of the interactions between nutrition, health, the gut, and productivity have grown, these two spheres of influence have begun to increasingly collide and integrate in the field. As nutritionists increasingly concern themselves with concepts like gut health, it is important to keep in mind that they approach these topics from a different starting point and set of expectations. It is therefore important to present the new complex data set, including measures like microbiome, metabolome, histology and gene transcription abundance in a way that is both intelligible, but more importantly, that is actionable. The objective of this talk is not necessarily to present new, ground breaking data, but more to help you translate your own new and ground breaking data into a format that can clearly communicate the potential impact it can have when used as the basis for change.

**Key Words:** Poultry, microbiome, visualization

**320S  Combining microbiome, gene copy, and metabolomic pathway in the analysis: A multi-omics case study.** Bill Shannon*, BioRankings, St. Louis, Missouri, United States.

In this presentation, we are interested in answering the question of whether gut bacteria impact insulin sensitivity/resistance in humans through the methionine degradation pathway. Using methods from ecology, we
modified RLQ to combine microbial taxa read counts, gene copy numbers for the taxa, and subject covariates to answer this question. While our example is from the HMP2 pre-diabetes overfeeding study examining the transition from insulin sensitive to resistance, the method is applicable to all animal health microbiome studies where there is interest in discovering possible metabolic mechanisms related to health and outcomes. In our example, more copies of KEGG K01251 and K00558 genes were associated with insulin sensitivity, and fewer copies with resistance. This leads to a testable hypothesis: Could increasing taxa with more K01251 and K00558 decrease conversion to insulin resistance? This presentation will show how to format the data and run analyses to generate testable hypotheses for any gut health problem. Replacing insulin S/R with health/performance traits of interest, RLQ can be used to develop microbial products to (1) decrease NE in chickens, (2) increase milk production in cattle, (3) fight viral diseases in pigs, or (4) reduce colic severity in horses.

**Key Words:** RLQ, multi-omics, biostatistics
Market, Welfare and Nutritional Dynamics of the Laying Industry: How the Layer Industry has Changed, Where it is Today, and Where it May be Going


The presentation will be divided into 3 sections: In the first section, the key market dynamics over the past 20 years will be reviewed: growth in production (global and region-specific), the importance of Asia, the impact of the ban of conventional cages in Europe, longer production cycles, the new appreciation for the nutritional value of eggs and the growth of the further-processed egg segment. In the second part, we will review the influence that the new generations of consumers, mainly the Gen Z, will have on the egg market over the next 10 years. In addition to price, taste and convenience, these new consumers are looking for foods that meet their demands in terms of sustainability and authenticity. The research and development of new products and the marketing strategies must focus on the needs of younger consumers. The final section of this presentation will consider some of the implications that the COVID19 pandemic may have on the egg business over the next few years in terms of production, consumption and types of products.

Key Words: Eggs, consumer trends, Gen Z, marketing

322S New insights on eggshell mineralization and how they can contribute to maintain shell quality. Joel Gautron*1, Lilian Stapane1, Alejandro Rodriguez-Navarro1, Yves Nys1, M.T. Hincke4, 1INRAe, Nouzilly, France, 2Avian Biology & Poultry Research unit, Université de TOURS, Tours, France, 3University of Granada, Granada, Spain, 4University of Ottawa, Ottawa, Ontario, Canada.

The eggshell is a critical barrier against mechanical stresses, dehydration and microbial penetration. Its integrity is essential to maintain the hygienic quality of this basic human food and to limit the number of downgraded eggs. In such a context, we are looking for eggshell strength specific markers in order to optimize egg quality. The eggshell is made of 95% mineral phase (calcium carbonate in calcite form) and an organic matrix (3.5%) mostly containing proteins. Eggshell formation arises from an extra-cellular mineralization process occurring in the uterus, in a fluid that contains eggshell precursors and involves a transient phase of amorphous calcium carbonate (ACC). Shell mineralization requires a large and continuous supply of calcium that comes in part from the diet and in part from the skeleton (medullary bone). Medullary bone serves as a calcium reservoir for eggshell calcification during the night and its resorption and formation synchronized with eggshell formation. In this review, we will describe both mineral supply and biomineralization mechanisms. We will focus on recent knowledge on vesicular transport to stabilize ACC and to address it to the mineralization site. This model of calcification using vesicles to stabilize ACC, explained the fast deposition of the crystalline calcite-oriented layer in the shell. The proteins described are currently further explored as biological markers. Their abundances relative to the quality of shell, age of birds (long laying cycles) and vitaminic status are explored. Genes coding these proteins are currently mapped on chicken genome for a selection of chicken layers with improved mechanical properties.

Key Words: eggshell, mineralization, eggshell quality


Animal Welfare requirements impose challenges to farmers, bringing additional cost that make it more difficult to run a profitable business. For this reason farmers used to see laying hen welfare requirements as a threat. In recent years changes have taken place. From an egg producer with a defensive attitude farmers have evolved to managers with an awareness of consumer demands and a smart strategy to market animal welfare and turn it into a profitable selling point.

Key Words: poultry welfare, housing, management, laying hens, market opportunities


There have been tremendous advances in lowering CP in broilers. Although work in shell egg layers is not to the scale of broilers, recent work in broilers concerning lowering of CP, optimizing efficiency through amino acid nutrition, and using additional feed grade amino acids warrants attention in the layer industry. Our presentation will address changes in hens and techniques to manage her that require fine-tuning amino acid minimums. A look at past literature on amino acid responses in layers will be presented. In addition, formulation trends will be assessed with reference to sustainability. And finally, the rise of sustainable egg operations in the third world (e.g., One Egg) whereby vegetable protein for hens is providing essential amino acids in the form of eggs to diets of people in poverty-stricken areas will be discussed.

Key Words: laying hen, shell egg layer, amino acid

In the last four years, the proportion of eggs produced in cage-free housing (conventional and organic) in the United States (US) has increased from approximately 10% to 22% of the market. By 2025 it is projected that over 50% of eggs produced in US will be cage-free. In an industry that has been primarily caged since the 1960’s, this has necessitated a significant rethinking of how we manage the farms and birds. Breeds that perform well in cages do not necessarily perform well in cage-free housing, and vice-versa. Diseases such as Blackhead, which we once thought were broiler and turkey diseases, are now seen in layers. We now must manage litter instead of manure belts and pits, track floor eggs, and piling mortality. And finally, veterinarians and nutritionists have had to make changes to their health and nutrition programs for cage-free production. In this presentation, I will discuss the various housing types used and challenges associated with each, layer performance in caged and cage-free environments, and how nutrition programs are modified for cage-free production.

Key Words: laying hen, cage-free
Student Workshop: How to Be a Better Reviewer

326S How to be a better reviewer: Section Editor perspective. Wei Zhai*, Mississippi State University, Starkville, Mississippi, United States.
No description provided.
Key Words: reviewer, Section Editor

327S How to be a better reviewer: Reviewer perspective. Ryan Dilger*, University of Illinois, Urbana, Illinois, United States.
No description provided.
Key Words: reviewer

328S How to be a better reviewer: Publisher perspective. Diana Jones*, Tom Fitzpatrick1, 2Elsevier Inc., Sydney, New South Wales, Australia, 2Elsevier Inc., San Diego, California, United States.
No description provided.
Key Words: reviewer, publisher

Similarities and differences on table egg regulations from around the world will be shared.

Key Words: egg regulations


Different perspectives on table egg production around the world will be shared.

Key Words: egg production

331S Overview of table egg industry: current issues/concerns. Chad Gregory*, United Egg Producers, Johns Creek, Georgia, United States.

The US table egg industry is in a time of change. Issues and concerns related to various aspects of producing eggs will be presented.

Key Words: table egg

332S Regulatory bodies within the table egg industry – What is the scope of the agency? (FDA). Gerardo A. Ramirez*, U.S. Food & Drug Administration, College Park, Maryland, United States.

The involvement of federal regulatory agencies can be challenging for egg producers. The information presented will share the role of the regulatory agency in producing and marketing eggs.

Key Words: table egg

333S Regulatory bodies within the table egg industry – What is the scope of the agency? (USDA). Jeffrey Hendricks*, USDA-AMS, Washington, District of Columbia, United States.

The involvement of federal regulatory agencies can be challenging for egg producers. The information presented will share the role of the regulatory agency in producing and marketing eggs.

Key Words: table egg


The involvement of federal regulatory agencies can be challenging for egg producers. The information presented will share the role of the regulatory agency in producing egg products.

Key Words: egg products

335S Regulatory bodies within the table egg industry – What is the scope of the agency? Jeffrey Hendricks*, USDA-AMS, Washington, District of Columbia, United States.

The involvement of federal regulatory agencies can be challenging for egg producers. The information presented will share the role of the regulatory agency in producing egg products.

Key Words: table egg

336S Academic perspective – where are we? Darrin M. Karcher*, Purdue University, West Lafayette, Indiana, United States.

Laying hen, table egg and egg product research will be discussed that is being conducted within the US.

Key Words: academic perspective

337S Canada perspective – where are we? Douglas R. Korver*, University of Alberta, Edmonton, Alberta, Canada.

Laying hen, table egg and egg product research will be discussed that is being conducted within Canada.

Key Words: laying hen, table egg, egg product research


Laying hen, table egg and egg product research will be discussed that is being conducted within Europe.

Key Words: laying hen, table egg, egg product research
Strategies to Increase Recruitment and Engagement of a Future Poultry Workforce

339S Recruitment Strategies for the Next Generation of Poultry Workers. Jessica B. Wells*, Mississippi State University, Starkville, Mississippi, United States.

The presentation will discuss the ever-evolving nature of recruitment and how poultry science departments and industry will face new challenges with recruitment of the next generation of poultry students and workers. Recruitment is the forefront of ensuring the success of the poultry industry as well as the existence of University Poultry Science Departments nationwide. With changes in each generation pertaining to how to engage and communicate, it is important that recruitment strategies also change to meet the demands of each student population. Successful recruitment strategies can help in not only securing students into poultry departments, but also ensuring those students are the right fit for the department and therefore are more likely to be successful in the poultry industry.

Key Words: Recruitment, student retention, poultry science departments

340S Industry approaches to recruiting students and connecting with academia. Stacy Webb*, Sanderson Farms, Laurel, Mississippi, United States.

The presentation will discuss industry approaches to recruiting students and connecting with academia. As our industry continues to grow, we are all faced with recruiting challenges and a shortage of viable candidates. Embracing those challenges and focusing on certain approaches can absolutely impact recruiting successes. Whether you choose to recruit future employees with face to face interaction, connecting with academia, networking, social media, or a virtual meeting platforms, adapting to these strategies may be the difference between landing the potential candidate or losing them to another industry.

Key Words: Poultry Industry, Recruitment, Student Recruitment

341S Engaging the Next Generation of Poultry Professionals. Elizabeth L. Karcher*, Purdue University, West Lafayette, Indiana, United States.

The number of incoming animal and poultry science students with no little or no experience with food animal production continues to grow. First year students arrive on campus with a limited awareness of careers and job opportunities in both animal and poultry sciences. Introductory courses provide opportunities to increase interest and curiosity in animal production early in a student’s undergraduate program. This presentation will focus on evidence-based classroom instructional strategies to develop student interest and motivation by supporting needs for autonomy and relatedness.

Key Words: Undergraduate, Engagement, Interest, Curiosity, Education

342S Innovative Uses of Technology to Recruit and Reach Students. Marissa Herchler*, North Carolina State University, Raleigh, North Carolina, United States.

With the increased demand in distance education for non-traditional students, use of innovative technology in agricultural disciplines is needed to supplement traditional teaching methods to keep students engaged. The NC State Feed Milling program has utilized a number of new techniques and technologies in feed mill design, feed formulation, virtual reality, and augmented reality over the previous two years to increase student engagement in synchronous and asynchronous courses. This presentation will focus on the development and implementation of these new technologies and their use in the classroom and Extension.

Key Words: distance education, virtual reality, augmented reality
Bone Health and Lameness, the Tip of the Iceberg

343S The development of osteochondrosis and tibial dyschondroplasia and the role of trace minerals and vitamins. Laura Amundson*1, 2, 'Zinpro Corporation, Eden Prairie, Minnesota, United States, 'University of Wisconsin, Madison, Wisconsin, United States.

Skeletal unsoundness, ultimately leading to lameness, early culling, and major financial hardships in production livestock, is a problem that continues to plague agriculture. Chondral dysplasia disorders such as osteochondrosis (OC) and tibial dyschondroplasia (TD) are cartilage defects that lead to disruptions in endochondral ossification, the tightly regulated processes coupling cartilage and bone formation with turnover. While both disorders have been persistent problems in production agriculture systems such as poultry and swine, the etiology of the diseases remains elusive. The rate limiting step of endochondral ossification is the modeling and remodeling of the extracellular matrix. The matrix metalloproteinases (MMP) and vascular endothelial growth factor (VEGF) have been implicated in cartilage, bone, and joint disorders where these factors are usually upregulated. The MMPs, a group of zinc dependent enzymes responsible for the degradation of matrix proteins during normal and aberrant growth, are functional in early embryonic development. Although OC and TD can both develop from multiple factors, the lack of a predictable animal model to study the initiation and progression of these cartilage lesions limits the detection and treatment of the disorder before acute lesions develop. We have developed a hypovitaminosis D kyphotic swine model that displays cartilage and bone abnormalities consistent with OC-like lesions in the vertebrae and femurs of these animals. The MMP and VEGF genes have been evaluated in this model and expression is regulated by nutritional inputs such as vitamin D, Ca, P, Zn, and Mn. This model will be useful and pertinent to identifying initial perturbations in pivotal cell signaling pathways that lead to uncoupled endochondral ossification, retained cartilage, and ultimately OC and TD. Understanding these mechanisms will greatly benefit poultry, swine, and other production animal industries.

Key Words: Vitamin D, Zinc


Until recently, infectious lameness in broilers was usually considered to be of bacterial or viral origin. If not infectious, it was an issue with the diet. Interesting to note, Staphylococcus arthritis has been a common sequela to coccidiosis in broiler breeder pullets. As the poultry industry has moved to less use of antibiotics, including the ionophore anticoccidials, we have begun to see more infectious lameness in broilers. We now understand that the bacteria associated with many of these infectious lameness issues in broilers are part of the intestinal normal bacteria flora. Success in the no use of antibiotics era of production is going to require maintenance of intestinal health and integrity to insure these normal flora bacteria stay in the intestines and don’t find their way into the broilers blood stream to localize in joints and bones. Maintaining gut health by minimizing the negative impact of coccidia or exposure to mycotoxins or biogenic amines or other causes of insult to the intestines becomes critical to reducing lameness in no antibiotics ever broiler production. In addition to preventing infectious lameness, maintaining intestinal integrity to insure adequate absorption of vitamins and nutrients becomes more difficult in a no antibiotics ever program.

Key Words: Infectious Lameness, Broilers, Normal Flora, Coccidiosis


The work to be presented focuses on improving our understanding of some of the potential interactions between phytate source and concentration as well as calcium (Ca) sources and particle size on standardized ileal digestibility (SID) of Ca and phosphorus (P) in diets with or without phytase. 1. From work comparing Ca sources (limestone vs bone meal at the same particle size) Ca source has a profound effect on SID Ca and P. Based on solubility of these two sources, Ca reactivity (solubility at low pH) may be the most important factor involved in the phytate Ca interactions. 2. Phytate source impacts both SID Ca and P.
Two phytate sources, corn or SBM were used at the same concentration (2.3 g/kg phytate P) if phytate P (PP) and SID Ca and P in diets with the same concentration and source of limestone differed. This suggests that as diets are formulated closer to requirements, phytase matrix values should start to reflect the effect of phytase on the specific phytate containing ingredients used in the diet. That is, phytase matrix should vary with formula ingredient use. 3. Particle size of limestone has a profound effect on SID Ca of limestone and SID P of the diet the limestone is added to, both in the presence or absence of phytase. Updated Ca, non phytate P, digestible Ca and P recommendations will be shared that focus on the impact of Ca and P on performance and bone mineralization.

Key Words: Calcium, Phosphorus, Digestibility, Broiler, Requirements

347S How feed additives influence lameness. Adnan K. Alrubaye*, University of Arkansas, Fayetteville, Arkansas, United States.

We have determined that Zinpro Availa-ZMC is effective in reducing the incidence of lameness by 20-25% (P<0.05) in broiler trials when using experimental models to induce lameness. Bacterial Chondronecrosis with Osteomyelitis (BCO) lameness is a major animal welfare and food safety issue that affects the commercial broiler industry in the United States and many other countries across the World. Lameness due to BCO is caused by a variety of pathogenic microorganisms as well as stress factors, which degrade the growth plates of the proximal and distal femoral and tibial heads. Our work demonstrates that bacteria leak across epithelia of the respiratory and gastrointestinal systems into the bloodstream. Bacteria that colonize the growth plates lead to necrosis leading to an inability to stand or walk due to pain resulting from severe damage. Thus, BCO is considered a major animal welfare issue where outbreaks in broiler operations may reach lameness of 10%. There are many factors that can stress birds like rapid growth, handling, transportation, heat, and respiratory infections, leading to increased BCO lameness. We have used the wire flooring model developed by Dr. Wideman, Department of Poultry Science at the University of Arkansas, to experimentally create stress in order to induce lameness. We have developed a bacterial challenge with Staphylococcus agnetis, that mimics the lameness epidemic for birds on litter flooring. Challenged birds will experience lameness at 70%, while the unchallenged birds in the same facility experience BCO lameness at 50%. The litter flooring with S. agnetis challenge is a better system for evaluation of epithelial integrity and immune function, using conditions that mimic real field conditions. The Availa-ZMC improved gut integrity by strengthening the tight junctions in the epithelia reducing bacterial leakage, enhanced bactericidal activity of adherent peripheral blood monocytes, and reduced overall lameness. Improving gut health, strengthening tight junctions, and improving bone strength are all key to preventing BCO lameness and improving the animal welfare of broilers.

Key Words: Broiler, Lameness, Chondronecrosis, Staphylococcus, Organic trace mineral